From the Department of Neuroscience
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ON THE EVOLUTIONARY ORIGIN OF THE VERTEBRATE CORTEX

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On the cover: Photomicrograph of the three-layered primordial cortex of the lamprey. In green are the IT-type neurons and in red are the GABAergic neurons. The outer molecular layer has fibres and is largely devoid of cells. The outer cellular layer has a larger proportion of excitatory cells, while the inner cellular layer has a larger proportion of GABAergic cells.

On the back: Morphology of a thalamo-recipient (neocortical layer 4-equivalent) cell of the lamprey cortex. The spiny dendrites extend to the molecular layer with significant ramifications.

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On the evolutionary origin of the vertebrate cortex

THESIS FOR DOCTORAL DEGREE (Ph.D.)

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To the pursuit of knowledge

|| न हि ज्ञानेन सदृशं पवित्रमिह विद्यते ||

[*nahi gnanena sadrusham pavitramiha vidyate*]

“There is nothing in this world that can purify the mind as knowledge”

Bhagvad-Gita, Chapter 4, Verse 38 (Circa 200 BCE)

ABSTRACT

The aim of this thesis is to dissect out the minimalistic ancestral neural hardware, which has been conserved in homologue structures of the cerebral cortex and in particular the neocortex, across vertebrates. For this purpose, we utilise the *lamprey* - a cyclostome (jawless vertebrate), and we compare its anatomical homologue of cortex, the lateral pallium (LPal), in terms of its connectivity, cytoarchitecture and sensory organisation with that of the mammalian neocortex. The lamprey has an important phylogenetic position as a basal and most ancestral vertebrate group that diverged from the main vertebrate line of evolution around 560 million years ago. The dissection of the homologue cortical structures in lamprey will allow us to understand the ancestral design of cortex in the common vertebrate ancestor, consequently contributing to develop a potential pan-vertebrate schema for cortical evolution.

In Paper 1, the role of lateral pallium in generation of different types of movements, as well as its efferent projection pattern to downstream motor centres were investigated. The results showed that the lamprey LPal mirrors the efferent projection pattern seen in the neocortex in mammals, with projections to the basal ganglia nuclei, optic tectum, mesencephalic tegmentum and to the rostral spinal cord. Electrical stimulation of the LPal generated well-defined movements of the eyes, mouth, body as well as locomotion, demonstrating the presence of a motor area. Following this in Paper 2, the cytoarchitecture and the microcircuit was examined and the LPal was shown to be a three-layered cortex and consist of similar proportions of excitatory neurons and GABAergic neurons as the mammalian cortex. The LPal also had equivalent functional cell types - the efferent projection neurons similar to neocortical layer 5b cells (PT-type), the thalamo-recipient neurons as layer 4-like cells and the intratelencephalic (IT)-type neurons. The results indicated the presence of a basic input-output microcircuitry reminiscent of the mammalian neocortex. In Paper 3, the organisation of visual and somatosensory input was examined. The lamprey as other anamniotes, was thought to have largely olfactory processing in the “primitive” pallium. Our investigations showed that the lamprey LPal/cortex indeed had a visual area with a retinotopic organisation, as well as a somatosensory area with distinct representations of the head and body, all relayed via the thalamus. There is thus a motor area and separate visual and somatosensory areas in the lamprey cortex. In paper 4, we examined the afferent and efferent connectivity of the dopaminergic substancia nigra *pars compacta* (SNc) which was virtually identical to that seen in mammals, including a strong input from the LPal.

The overall results of the four studies demonstrated an unforeseen level of conservation of the basic microcircuitry, cytoarchitecture, cell-types, efferent connectivity and sensorimotor organisation of the lamprey LPal/cortex providing compelling evidence for common ancestry with the mammalian neocortex. This leads to the overarching conclusion that the basic features of the cortex, and when taken in tandem with previous extensive subcortical homologies demonstrated, the basic design of the forebrain, had already evolved at the dawn of vertebrate evolution.

LIST OF SCIENTIFIC PAPERS

I. Ocana, F.M*., **Suryanarayana, S.M***., Saitoh, K., Kardamakis, A.A., Capantini, L., Robertson, B., Grillner, S. (2015) The lamprey pallium provides a blueprint of the mammalian motor projections from cortex. Curr. Biol. 25: 413–423.
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II. **Suryanarayana, S.M**., Robertson, B., Wallén.P., Grillner, S. (2017). The lamprey pallium provides a blueprint of the mammalian layered cortex. Curr. Biol. 27: 3264-3277.

III. **Suryanarayana, S.M**., Pérez-Fernández, J., Robertson, B., Grillner, S. The evolutionary origin of visual and somatosensory representation in the cortici of vertebrates. *Manuscript*

IV. Pérez-Fernández, J., Stephenson-Jones, M., **Suryanarayana, S.M**., Robertson, B., Grillner, S. (2014) Evolutionarily conserved organization of the dopaminergic system in lamprey: SNc/VTA afferent and efferent connectivity and D2 receptor expression. J. Comp. Neurol. 522: 3775–3794.

LIST OF SCIENTIFIC PAPERS NOT INCLUDED IN THESIS

I. **Suryanarayana, S. M**., Hellgren Kotaleski, J., Grillner, S., Gurney, K. N. (2019) Roles for globus pallidus externa revealed in a computational model of action selection in the basal ganglia. Neural Netw 109:113–136.

II. Olson, I., **Suryanarayana, S.M**., Robertson, B., Grillner, S. (2017) Griseum centrale, a homologue of the periaqueductal gray in the lamprey. IBRO Rep. 2: 24–30.

III. Robertson, B., Kardamakis, A., Capantini, L., Pérez-Fernández, J., **Suryanarayana, S. M**., Wallén, P., Grillner, S. (2014) The lamprey blueprint of the mammalian nervous system. Prog. Brain Res. 212: 337–349.

CONTENTS

LIST OF ABBREVIATIONS

CNS	Central nervous system
CPG	Central pattern generator
DCN	Dorsal column nucleus
dmtn	Dorsomedial telencephalic nucleus
DVR	Dorsal ventricular ridge
EPSP	Excitatory postsynaptic potential
GABA	γ-aminobutyric acid
GPe	Globus pallidus *externa*
GPh	Globus pallidus projecting to habenula
GPi	Globus pallidus *interna*
Hb	Habenula
Hyp	Hypothalamus
IPSP	Inhibitory postsynaptic potential
IT	Intratelencephalic tract
LFP	Local field potential
LGN	Lateral geniculate nucleus
LOT	Lateral olfactory tract
LPal	Lateral pallium
MLR	Mesencephalic locomotor region
MPal	Medial pallium
MRRN	Middle rhombencephalic reticular nucleus
nII	Optic nerve
NTP	Nucleus of the posterior tuberculum
OB	Olfactory bulb
ot	Optic tract
OT	Optic tectum
Pi	Pineal organ

PO	Preoptic nucleus
PT	Pyramidal tract
Rh	Rhombencephalon
SNc	Substantia nigra, *pars compacta*
SNr	Substantia nigra, *pars reticulata*
STN	Subthalmic nucleus
Str	Striatum
Th	Thalamus
VTA	Ventral tegmental area

1 INTRODUCTION

"There is grandeur in this view of life, with its several powers, having been originally breathed into a few forms or into one; and that, whilst this planet has gone cycling on according to the fixed law of gravity, ***from so simple a beginning endless forms most beautiful and most wonderful have been, and are being, evolved****"*

- Charles Darwin
On the Origin of Species (1859)

A seminal step forward in the furtherance of scientific thought has undoubtedly been the proposal of evolution via natural selection (Darwin, 1859). It offered a radical and elegant explanation to the long-standing problem of the origin of the extraordinary variety of life on the planet through the proposal of descent from a *common ancestor*. It overthrew transcendental ideas of the Victorian era, and essentially seeded the modern biological thought process. This was also aptly timed, following the Geoffrey-Cuvier debates (1830) which was a clash between a common plan or 'archetype' for all animals as proposed by Geoffrey, with Cuvier's argument of several different plans (Ruse, 1989; Russel, 1916; Striedter, 2006). Darwin's theory supported the idea of a common plan and the realisation grew that animals evolve, and so do their brains. Although there were debates regarding the uniqueness of the human brain and doubts on Darwinian evolution (Huxley, 1880; Rupke, 1994), descent from a common ancestor as a theory soon gained a firm foothold and mainstream acceptance in the scientific community.

1.1 EVOLUTION OF THE NERVOUS SYSTEM

While the theory of natural selection clearly implied that the nervous system also evolved, it outlined the necessity of the examination of brains across species. Before the development of histological procedures, examinations of brains were largely focused on the gross anatomy. However, the advent of the Golgi stain (Golgi, 1873) and its subsequent use by Santiago Ramon y Cajal (Cajal, 1899; Ramón and Cajal, 1904; y Cajal and Muñoz, 1928), represented yet another seminal step forward in research into the evolution of the nervous system. The staining with detailed morphological characteristics of neurons and cytoarchitecture, allowed more insightful histological investigations of brains across species and resulted in the rapid development of the field of comparative neuroanatomy. Interest also arose in the brains of "lower" vertebrates with the view that their study would shed light on the evolution of vertebrate brains (Herrick, 1948) Evolution was thought to be essentially linear and was represented with the phylogenetic scale (Northcutt, 2001; Ruse, 1989).The generic view was that the "simpler" brains of "lower" vertebrates progressively evolved to the more "complex"

mammalian brains. This was particularly thought to be true in relation to the telencephalon - with the simplest telencephalic structure in the amphioxus with a single vesicle, with paired olfactory structures evolving in lower vertebrates, and with thalamic and non-olfactory modalities "invading" and only represented in the telencephalon of amniotes (reptiles and mammals; (Crosby, 1992; Herrick, 1948; Papez, 1929). Of course, this conceded that every species also evolved independently. This view although doubted and challenged (Northcutt and Wicht, 1997; Wicht and Northcutt, 1998), has more or less remained in the mainstream scientific thought due to lack of data. The results presented in this thesis unequivocally negates this point of view of a linear evolution from olfactory telencephalic centres in "lower" vertebrates to multimodal sensory processing in "higher" vertebrates with direct evidence, consequent of the investigations of the lamprey telencephalon in general, and the pallium in particular.

1.2 PHYLOGENTIC IMPORTANCE OF CYCLOSTOMES

The investigations of brain structures across species is thus a vital aspect of evolutionary neuroscience. The choice of the model organism for this thesis is the lamprey, a cyclostome. Cyclostomes and mammals represent two extreme groups phylogenetically (Figure 15). The former belongs to the earliest group of vertebrates which diverged from the main vertebrate line of evolution over 560 million years ago (Kumar and Hedges, 1998). There are two extant species of cyclostomes - the hagfish and the lamprey (Heimberg et al., 2010; Kuraku et al., 1999; Miyashita et al., 2019). The lamprey has an additional advantage in relation to the hagfish in that it has a well-developed visual system and fewer degenerate features when compared to gnathastomes (jawed-vertebrates) (Northcutt and Wicht, 1997; Suzuki and Grillner, 2018). The examination of brain structures in cyclostomes would in essence, help shed light on the ancestral organisation of brain structures from which all extant vertebrates (cyclostomes and gnathastomes) have ultimately evolved. Thus, phylogenetically cyclostomes have an important position.

1.2.1 Lamprey – an evolutionary pioneer

The lamprey in particular, has been pivotal in evolutionary research and its nervous system essentially constitutes a generic blueprint of the vertebrate brain (Grillner and Robertson, 2016; Robertson et al., 2014). It has been a model organism used extensively in research on motor control and in dissecting the neural circuits underlying movement, with the CPGs in the spinal cord being elucidated in detail (Grillner, 2006). The CPGs can be activated by the reticulospinal neurons which in turn, can be activated from the diencephalic and mesencephalic locomotor regions (DLR and MLR, respectively) (Brocard et al., 2010; Dubuc et al., 2008; El Manira et al., 1997), all of which are well-conserved structures across vertebrates. Electrical stimulation of the DLR/MLR activates the reticulospinal neurons and generates locomotor behaviour (Brocard et al., 2010; El Manira et al., 1997). Furthermore, in both lamprey and mammals a subpopulation of reticulospinal neurons can terminate locomotor behaviour (Bouvier et al.,

2015; Juvin et al., 2016). The reticulospinal neurons can also be activated from brainstem regions, including the optic tectum (homologue of the mammalian superior colliculus) and the pretectum, both of which have also been studied extensively in the lamprey (Capantini et al., 2017; Kardamakis et al., 2016; Kardamakis et al., 2015).

Moving to the forebrain, several studies established the presence of the basal ganglia subnuclei and demonstrated their conservation to a surprisingly detailed degree (Ericsson et al., 2011; Ericsson et al., 2013a; Grillner and Robertson, 2016; Perez-Fernandez et al., 2014; Robertson et al., 2012; Stephenson-Jones et al., 2012; Stephenson-Jones et al., 2013; Stephenson-Jones et al., 2011). With regards to the cortical homologue, the pallium in the lamprey had been considered largely to be olfactory with little or no non-olfactory modalities represented (Daghfous et al., 2018; Northcutt and Wicht, 1997). The evolutionary relationship of the pallium with the mammalian cortex was virtually unknown other than it being identified as the anatomical homologue with its subdivisions (Nieuwenhuys, 1997; Northcutt and Wicht, 1997). Given the high degree of conservation of the lamprey brain, we thought it prudent to examine the organisation of the lamprey pallium with anatomical, physiological and functional means in relation to the evolutionarily most recent region of the vertebrate brain, the cortex.

1.3 COMPARING THE LAMPREY LPAL WITH THE MAMMALIAN NEOCORTEX

We have in this thesis, compared the features of the mammalian neocortex with that of the anatomical homologue region of cortex in the lamprey. The lamprey pallium has two main anatomical subdivisions, the medial pallium (MPal) and the evaginated portion as the lateral pallium (LPal). The MPal is considered to be the anatomical homologue of the hippocampus while the LPal is thought to be the cortex (Nieuwenhuys, 1997; Northcutt and Wicht, 1997). We examine here the LPal, using electrophysiological, neuroanatomical and immunohistochemical methods.

The mammalian neocortex is a six-layered structure with distinct motor and sensory areas. Amongst the sensory areas, one can notice a striking specificity of modality from the relays in thalamus, which are the major source of relayed sensory input targeting layer 4 of the neocortex. For instance, the visual cortex receives visual input from the lateral geniculate nucleus of the thalamus. Furthermore, the visual input has a topographic mapping in the visual cortex with a well-defined retinotopy (Connolly and Van Essen, 1984; Redgrave et al., 2010; Tootell et al., 1982). A parallel visual pathway relays processed visual information from the superior colliculus via the lateral posterior nucleus of thalamus to the prestriate visual cortex (Dugas-Ford and Ragsdale, 2015; Schneider, 1969). A similar sensory channel exists for the somatosensory input, which via the dorsal column nuclei (DCN) reaches the ventrobasal nucleus of the thalamus and is then relayed to the somatosensory cortex. The somatosensory input is also mapped with a topography, the *homunculus* (Johnson, 1990; Mountcastle, 1984). Similar distinct sensory areas also exist for other sensory modalities. The sensory areas send descending projections from layer 5b neurons to downstream motor centres like the superior

colliculus and the brainstem. Other than the sensory areas, there is a distinct primary motor area of cortex, which sends efferent projections to downstream motor centres in the midbrain, brainstem and to the spinal cord (Lemon, 2008).

Our data shows that the lamprey LPal is a three-layered cortex with distinct visual, somatosensory and motor areas and an efferent projection pattern characteristic of the mammalian neocortex, and in addition, the sensory thalamocortical relay. The motor area can generate well-delineated movements of the eye, mouth, body and locomotion. Distinct glutamatergic projection neurons monosynaptically target different downstream motor centres, while another subpopulation projects intratelencephalically targeting the ipsilateral striatum, and the contralateral cortex and striatum. These are equivalent to the layer 5b and 5a neurons, respectively. There is in addition, a separate subpopulation receiving thalamic input akin to the layer 4 neurons of the neocortex. The proportion of GABAergic (around 20%) and non-GABA neurons in lamprey is similar to that of the neocortex with GABAergic subtypes including calretinin- and calbindin-expressing cells. With regard to sensory areas, the visual cortex of the lamprey is retinotopically organised, with topography relayed from the thalamic visual relay area. The somatosensory cortex has distinct representations of the head and trunk. We also show that processed information of the body from the DCN is relayed via the thalamus. In addition, information from the head is relayed via the trigeminal nerve to a trigeminal sensory nucleus, which projects to thalamus. The thalamus has distinct neurons targeting the different sensory areas in the cortex. The overall evidence thus shows remarkable similarities with the neocortex and provides a compelling account of conservation of these features, thereby suggesting a common ancestry. Furthermore, as demonstrated previously, there is also a significant level of conservation of the basal ganglia, the dopamine system and the habenula (reviewed in Grillner and Robertson (2016). Given this surprising degree of similarities in both the cortical and subcortical structures, it seems highly unlikely that they have evolved through convergence. We therefore propose, that important parts of the vertebrate forebrain mediating decision-making and action selection, had already evolved at the dawn of vertebrate evolution when the first group of vertebrates diverged from the vertebrate evolutionary line leading to mammals more than 500 million years ago.

1.4 HOMOLOGY AND THE NEOCORTEX

Amongst different areas of the mammalian cortex, the six-layered neocortex is cytoarchitecturally the most recent. Its evolutionary ancestry has remained a bone of contention for more than a century (Northcutt, 2011; Northcutt and Kaas, 1995; Puelles, 2001). Distinct sensory and motor areas of the neocortex have been mapped across mammals and is generally agreed to be present in the last common ancestor of mammals (Brodmann, 2006; Kaas, 2013; Raghanti, 2017). However, when we move to non-mammals, defining homologies has been anything but straightforward. This has been due to the unusual plasticity seen in the evolution of pallia across the vertebrate lineage (Briscoe and Ragsdale, 2019). Unlike the neocortex, its

reptilian counterpart, the dorsal cortex is three-layered and is known to have a visual area albeit with a non-retinotopic representation (Fournier et al., 2018). Little is known about the cytoarchitecture and connectomics of homologue cortical areas in amphibians and teleosts, and much less so in cyclostomes. Over the years, the debate on neocortical homology has been based on several lines of evidence, which we briefly summarise below, and has centered largely on the reptilian ancestry of the mammalian condition (Briscoe and Ragsdale, 2018a).

1.4.1 What defines homology

Homology in the broadest sense has been described as "same organ in different animals under every variety of form and function" (Owen, 1843). It is now generally agreed that homology relates to *similarities* which arise in two structures due to *common ancestry* (Louise, 1984; Wagner, 1989; Weiss, 1994). Evidently, when dealing with complex cytoarchitectural structures such as the cortex, one can define homology by examining similarities at different levels. For instance, homologs can be identified during development as distinct conserved domains, since it is now well established that all vertebrate brains show surprising similarity during early developmental patterning (Puelles, 2001, 2017; Puelles et al., 2000; Puelles and Rubenstein, 2003). Homologues can be identified based on the similarities in cytoarchitecture and connectomics of the adult structures (Karten, 1969, 2015; Shepherd, 2011). One can also define cell-type homology based on similar molecular identity of specific cell groups (Briscoe et al., 2018; Briscoe and Ragsdale, 2018b; Dugas-Ford et al., 2012; Tosches and Laurent, 2019; Tosches et al., 2018). It is important to understand that there may not *always* be congruence at one level of homology with another. In general, however, corroborative evidence of similarities from different levels, strengthens the argument for common ancestry. We discuss below the prevailing ideas and evidence in defining cortical homologues across vertebrates.

1.4.2 Developmental pallial sectors

One major line of evidence in identifying cortical homologues has been the segregation of the developing pallium of embryos into distinct areas delineated with specific marker expression. These divisions have been refined with time from the tripartite model (Striedter, 1997) to the currently prevailing updated tetrapartite model (Puelles, 2017). The updated tetrapartite model essentially proposes four divisions of the developing pallium - the first three being the medial, lateral and dorsal pallium which are all Emx 1- expressing regions, and which develop into the hippocampus, claustro-insular complex and the neocortex, respectively. The dorsal pallium also selectively expresses Emx 2. The fourth division is the ventral pallium which, forms the olfactory cortici and the amygdala. These divisions have been affirmed with the expression of claustrum specific marker Nr4a2 and the insular specific marker Cyp26b (Puelles et al., 2016).

With regards to cyclostomes, all major sections of the developing telencephalon with the pallial and subpallial regions, as well as the lateral and medial ganglionic eminence has been recently identified (Sugahara et al., 2016). However, the pallial area has a generic Emx 1 expression

and more specific subdivisions of the pallium such as the dorsal pallium have not been shown (Sugahara et al., 2017; Sugahara et al., 2016). The four divisions have been identified in the developing pallia of avians and non-avian reptiles, as well as mammals (Puelles, 2017; Watson and Puelles, 2017). However, there is much less information on these sectors in teleosts and amphibians (Ganz et al., 2014; Mueller et al., 2011).

1.4.3 Basic input output circuitry

Another recurrent theme in identifying neocortical homologues has been in defining a basic input-output circuitry in both avian and non-avian reptiles with equivalent cell types and connectivity to those found in the mammalian neocortex (Harris and Shepherd, 2015; Shepherd, 2011). Since early on, work from Harvey Karten (Karten, 1969, 2013, 2015) provided an influential schema for input-output homologous circuits in avians and mammals with thalamorecipient layer 4-equivalent cells, output layer 5b-like projection neurons and associational intratelencephalic layer 5a-like projection neurons. These equivalent cell types have been found also in the dorsal cortex of non-avian reptiles and pallial regions (DVR and Wulst) of avians and has been presented as an ancestral scheme that has been maintained from reptiles to mammals (Briscoe and Ragsdale, 2018a; Dugas-Ford and Ragsdale, 2015; Dugas-Ford et al., 2012).

1.4.4 Cell-type marker specificity

The question of neocortical homology has also been investigated with marker specificity of different cell types (Dugas-Ford and Ragsdale, 2015; Montiel and Aboitiz, 2018; Tosches and Laurent, 2019). This has been used to substantiate the conserved nature of the input-output circuitry proposed by Karten (1969). Through examination of select cell-type specific markers, equivalent cell types were described in the reptilian dorsal cortex and the mammalian neocortex, including layer 4-like thalamorecipient neurons, intratelencephalic neurons and layer 5-like projection neurons (Dugas-Ford and Ragsdale, 2015; Dugas-Ford et al., 2012). However, more recent high throughput sequencing investigations of the reptilian cortex have revealed an overlap of markers in cell types in the dorsal cortex, which are not specific to any single excitatory cell type in the neocortex (Tosches et al., 2018). On the other hand, there seems to be conservation of the GABAergic interneurons at least at the level of "classes" or the major subgroups of GABAergic interneurons in the reptilian dorsal cortex and the mammalian neocortex. Thus, it has been concluded that there are no "one-to-one" homologues of the excitatory cell types found in the mammalian neocortex in the reptilian dorsal cortex, while the GABAergic neuronal subpopulations have been more robust in their conservation and homology (Tosches and Laurent, 2019; Tosches et al., 2018) from a molecular marker expression perspective.

We introduce our aims and present our results of the lamprey LPal/cortex along with the discussion of the results. In addition, we have a general discussion where we cover the

evolutionary implications of our results and their impact on the ongoing debate regarding neocortical evolution. The more interested reader is referred to the appended Literature Review which covers pertinent literature regarding pallia across vertebrates – from anamniotes to the human neocortex.

2 AIMS

STUDY 1

- To examine whether the lamprey LPal/cortex has a motor area and whether it is able to generate different kinds of movements.
- To charecterise the efferent projection pattern of the LPal/cortex and compare it with efferent projections of the mammalian neocortex.

STUDY 2

- To examine the overall cytoarchitecture of the LPal and the proportion of GABA and glutamatergic cells.
- To examine the microcircuit of the LPal /cortex, including the physiology and morphology of different cell types.
- To study thalamic, intratelencephalic and olfactory inputs.

STUDY 3

- To examine representation of different sensory modalities in the LPal /cortex.
- To charecterise the topography of sensory representations in the lamprey cortex- retinotopy and somatotopy.
- To delineate sensory pathways relayed via the thalamus - visual and somatosensory.

STUDY 4

- To examine the afferent and efferent connectivity of the dopaminergic SNc in the lamprey.
- To examine the similarities of lamprey SNc connectivity with that of the mammals.

3 RESULTS AND DISCUSSION

3.1 PAPER 1: INVOLEMENT OF THE LATERAL PALLIUM IN GENERATING MOVEMENTS AND ITS EFFERENT CONNECTIVITY

In the first study (Ocana et al., 2015), we investigated whether the lamprey LPal was capable of generating motor behaviour, and examined its efferent projection pattern. The focus was on the evaginated part of the lamprey pallium, the LPal, which as explained earlier is the anatomical homologue of cortex of mammals (Northcutt and Wicht, 1997). Paper 4 included an important aspect of the efferent connectivity of LPal when looking at its afferent input to the dopaminergic substantia nigra *pars compacta* (SNc) (Perez-Fernandez et al., 2014).

3.1.1 Identifying a motor pallium

In mammals including man, the motor areas of cortex can elicit different types of movements of the eyes and body (Georgopoulos and Grillner, 1989; Lemon, 2019; Lemon, 2008), in response to electrical stimulations (Penfield, 1937). To examine whether a motor region was present in the lamprey LPal/cortex and whether electrical stimulations could elicit different types of movements, we used a semi-intact preparation of the lamprey (Saitoh et al., 2007) with the head fixed but with the body free to move (see Paper 1, Methods).

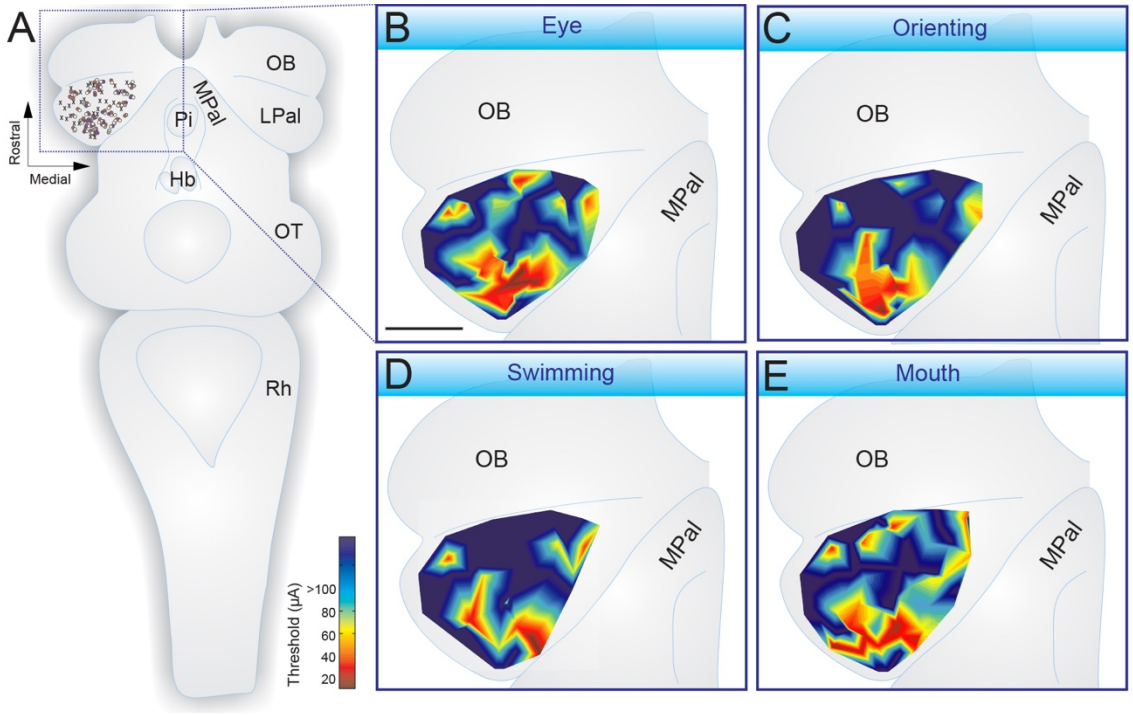

Figure 1. Heat maps in LPal for different movements. **A.** Schematic of the lamprey brain showing the stimulation sites in the LPal. Heat maps showing the stimulus intensity needed to elicit different movements **B**. Eye, **C**. Orienting, **D**. Swimming, **E**. Mouth. The dark colours indicate a lower threshold (minimal stimulation strength needed to evoke that particular type of movement).

Extracellular low-threshold electrical stimulation of the LPal were performed, which gave rise to movements of the eyes, neck, oral movements and locomotion (Figure 1A-E). These motor areas could be localised to caudolateral areas of the LPal, which can be identified as a "motor pallium". Different properties of elicited movements including amplitude, latency and duration were dependent on stimulus parameters. These movements were mediated by distinct subpopulations of glutamatergic projection neurons in the motor pallium which monosynaptically targeted different motor centres, as for instance the optic tectum and the mesencephalic tegmentum (discussed below, Figure 2).

3.1.2 Efferent connectivity of LPal

The next question we addressed was the efferent connectivity of LPal, to examine whether the different movements generated were due to monosynaptic glutamatergic projections, which is,

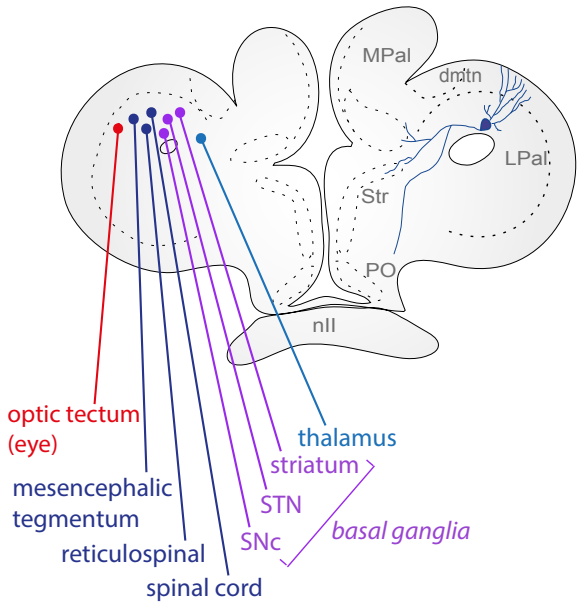

Figure 2. Efferent projections of the LPal/cortex. Schematic showing the efferent projection of the LPal. Distinct projections target all downstream motor centres, as well as the basal ganglia nuclei.

a typical aspect of mammalian neocortical control (Drew et al., 2004; Lemon, 2019; Lemon, 2008). We used anterograde tracing with injections of Neurobiotin in the LPal. The injections revealed efferent projections to all downstream motor centres, including the striatum, subthalamic nucleus, thalamus, deep layer of optic tectum, mesencephalic tegmentum, reticulospinal neurons and some fibres were observed interestingly also, at the level of the rostral spinal cord (Figures 2 and 3C-F). These efferent projections largely target the same areas as the equivalent projection neurons (layer 5b) in the mammalian neocortex. We established that the projections to the deep motor layer to the optic tectum were glutamatergic and monosynaptic through whole-cell patch recordings from tectal deep layer motor neurons and

extracellular stimulations of the LPal. The responses were abolished during bath applications of glutamate blockers. This projection is similar to the frontal eye-field projections to the deep layer of the superior colliculus. The responses from LPal stimulations were also abolished during bath application of glutamate blockers and intracellular recordings from the reticulospinal neurons, which as in mammals forms a fast disynaptic pathway to the spinal cord. Furthermore, in both cases, pharmacological tests along with reliability and latency of the elicited responses established that the responses were also monosynaptic. The location of these projection neurons in the LPal/cortex was an addition question. We addressed this with retrograde labelling from target motor areas, and we could map the projection neurons in the LPal and furthermore show through dual-tracer injections that the projections to distinct motor centres arose from distinct populations (indicated by colour code in Figure 2).

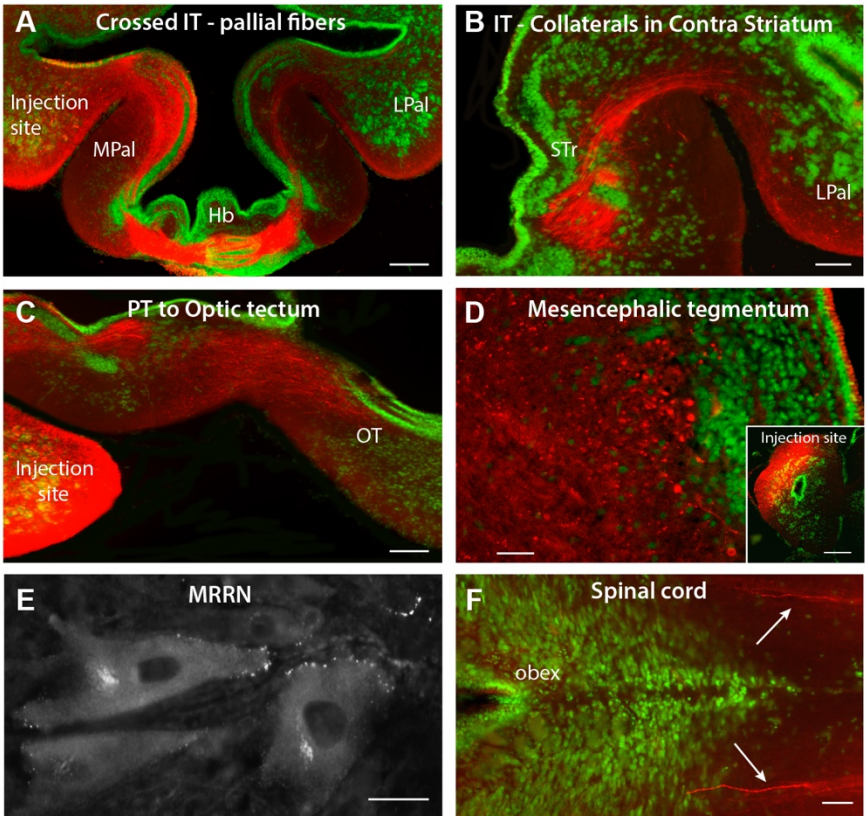

Figure 3. PT and IT projections. **A.** Photomicrograph of the lamprey telencephalon showing the IT fibres crossing in the habenula **B**. IT collaterals in the contralateral striatum **C**. PT fibres terminating in the optic tectum **D**. PT fibres in the mesencephalic tegmentum **E**. PT fibres targeting reticulospinal neurons **F**. Some PT fibres observed also at the level of rostral spinal cord just caudal to obex.

3.1.3 PT and IT projections

A major feature of the efferent glutamatergic projections of the mammalian cortex are the two major subdivisions - the "PT-type" or pyramidal tract/extratelencephalic projections, which target the different downstream motor centres and the "IT-type" intratelencephalic projections, which cross through the corpus callosum to target the contralateral cortex and striatum (Kim et al., 2015; Reiner et al., 2010; Suarez et al., 2014). The lamprey LPal/cortex also has these two

types of projections - the PT-type projections target the ipsilateral striatum and downstream motor centres while the IT-type cells target the ipsilateral and contralateral striatum and contralateral LPal (Figures 2 and 3). The IT fibres traverse through the medial pallium (MPal) to cross in the habenular commissure, continue in the contralateral MPal and terminate in the contralateral LPal while giving of collaterals in the contralateral striatum (Figures 3A, 3B).

3.1.4 Cortex has evolved as the "broadcaster" of motor commands

Electrical microstimulation has been used to identify motor areas in the mammalian cortex, including humans (Donoghue and Wise, 1982; Penfield, 1937). The mammalian motor cortex in addition has a homunculus representation in which different parts of the body are represented separately (Lemon, 2008) and complex motor behaviour can be elicited with longer durations of stimulation (Graziano et al., 2002). Although we could not ascertain whether there is an ordered mapping in the lamprey motor pallium, with higher intensity of stimulation a combination of movements like for instance those with both eye and orientation could be elicited. In summary, a motor area clearly existed in the lamprey LPal/cortex which could elicit different types of movements (Figure 1).

The presence of the detailed efferent projections in the lamprey LPal/cortex highlights the fact that the ancestry of cortical efferent control of movement has origins dating back to the dawn of vertebrate evolution. The efferent projection pattern in the lamprey LPal/cortex is virtually identical to that of the mammalian neocortex to the downstream motor areas (Lemon, 2019; Lemon, 2008). They are also glutamatergic and monosynaptic projections. In the neocortex, these projections arise from the layer 5b pyramidal neurons in primary sensory areas as well as motor areas, as for instance the projections of the frontal eye fields, motor cortex and the visual cortical projections to the superior colliculus (Fries, 1984). The cortical motor projections target all the major downstream motor centres. The corticospinal tract with its direct projections to the motoneurons in the spinal cord has been associated with the control of more dexterous and skilled movements in mammals but also in compensatory movements during locomotion and posture (Georgopoulos and Grillner, 1989; Lemon, 2019). The parallel downstream projections to the reticulospinal system from the cortex forming the disynaptic pathway to the spinal motor neurons is of importance in the control of movements (Fregosi et al., 2017; Lemon, 2008; Matsuyama et al., 2004). There is also evidence of the convergence of these parallel downstream pathways on spinal interneurons (Ortiz-Rosario et al., 2014).

The neocortex has been described as a "broadcaster" of motor command signals, which can utilise the subcortical motor infrastructure to generate and mediate goal-directed behaviours (Arber and Costa, 2018). Our data showing similar efferent connectivity in the lamprey LPal/cortex suggests that this was an ancestral design and that during evolution, this design has been maintained. Pallial projections to downstream centres have also been mapped in non-mammalian amniotes including reptiles and avians (See Literature Review, (Dugas-Ford and Ragsdale, 2015; Hall et al., 1977), as well as in anamniotes, as for instance in sharks, in which

pallial projections to the spinal cord has been observed (Ebbesson and Schroeder, 1971) (see Literature Review). These data further support the common ancestry of the cortical efferent projection pattern.

3.2 PAPER 2: CYTOARCHITECTURE AND MICROCIRCUIT BAUPLAN OF LPAL

In paper 2 (Suryanarayana et al., 2017), we looked at the cytoarchitecture, physiology and morphology of cell types and microcircuit features of the LPal/cortex including its afferent input. The results showed a three-layered cortex with similar functional cell types as seen in the mammalian cortici. The sensory input relayed from the thalamus was also characterised.

The LPal was shown to have an outer molecular layer largely devoid of neurons and a cellular layer, which could be subdivided into two parts based on the relative distribution of GABAergic neurons and general cell density. The "inner cellular layer" consists of a higher proportion of GABAergic cells and an "outer cellular layer" with relatively lesser proportion of GABAergic cells but a higher cell density than the inner cell layer (Figures 4A, B). The overall proportion of GABAergic neurons in LPal was about 22 % (Figure 4C), which is roughly the same proportion as in mammals. With regard to GABAergic interneurons, calbindin- and calretinin-expressing interneurons were present, as well as other GABAergic cells.

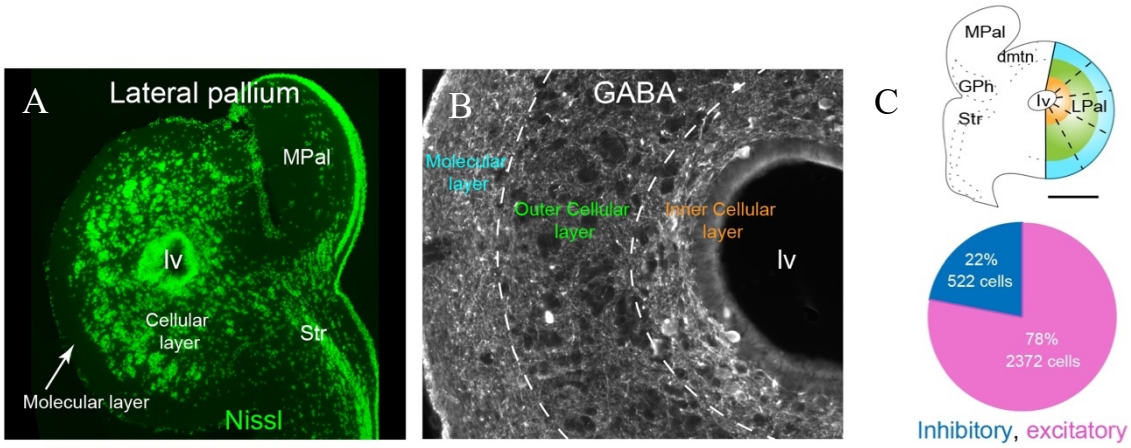

Figure 4. LPal/cortex lamination. **A.** Photomicrograph of a Nissl stained section of the lamprey LPal showing the molecular layer, the cellular layer and the ventricle. **B**. Photomicrograph of the LPal with a GABA immunostain showing the three layers, the molecular layer, the outer cellular layer and the inner cellular layer. **C.** Schematic showing the three layers in LPal and a pie-chart showing the proportion of GABAergic and non-GABAergic cells in the LPal.

3.2.1 Physiology and morphology of major excitatory cell types

To investigate the membrane properties and morphology of the excitatory projection neurons in LPal we performed injections in downstream target areas to retrogradely label the different projection neurons for electrophysiological recordings and intracellular labelling (Figure 5A).

Figure 5B shows the morphology of a PT-type projection neuron that targets the optic tectum. These neurons generally possessed two but sometimes one dendrite extending to the molecular layer. Like mammalian cortical projection neurons these dendrites are spiny (Figure 5C).

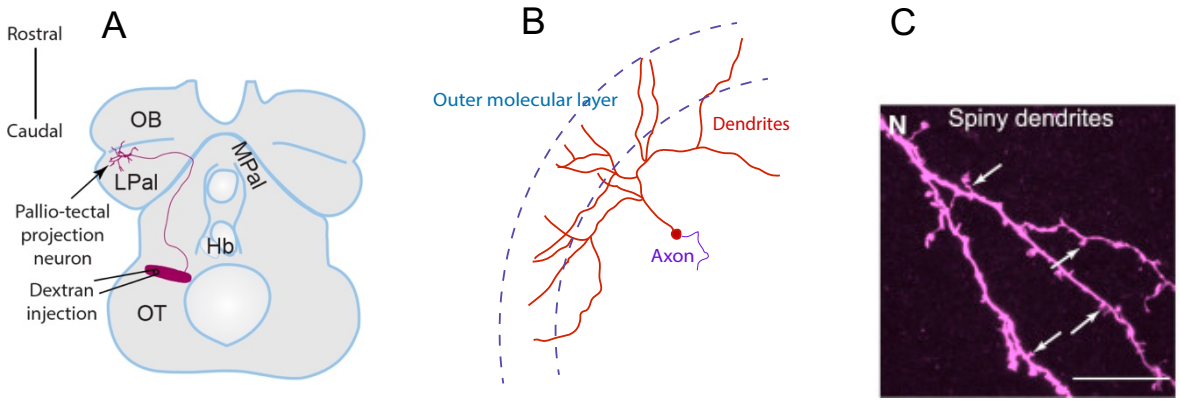

Figure 5. PT-Type cells. **A.** Schematic of the lamprey brain showing the injection of dextran rhodamine in the optic tectum to retrogradely label PT-type neurons for whole-cell patch clamp recordings. **B**. A reconstructed LPal neuron showing the dendrites extending to the molecular layer. **C**. Spiny dendrites of a LPal neuron.

Whole-cell patch clamp recordings revealed two classical cortical firing properties - regular and burst firing (Figures 6A and 6B). The morphology and electrophysiological properties of IT-type neurons were also characterised by prelabelling them for electrophysiological recordings.

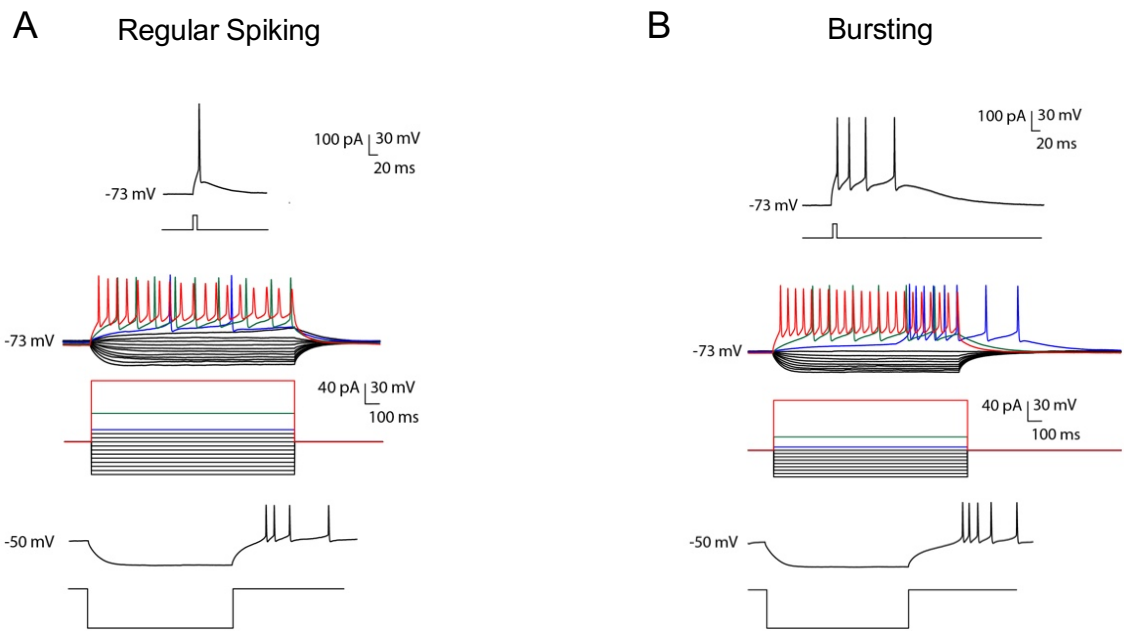

Figure 6. Firing properties of excitatory LPal neurons. **A.** Typical firing properties of a regular spiking LPal cell, the cell responds with a single spike after a short suprathreshold current injection and shows spike frequency adaptation for longer suprathreshold current injections. It also shows post-inhibitory rebound spiking **B.** Firing properties of a bursting cell, which responds with a burst of 3-4 spikes after a short suprathreshold current injection and shows spike frequency adaptation for longer suprathreshold current injections. It also shows post-inhibitory rebound spiking.

The IT-type cells also showed the typical two types of firing as the PT-type cells. They had spiny dendrites extending into the molecular layer as their PT-type counterparts. We additionally charecterised the morphology and firing properties of a third subtype of excitatory neurons in the LPal/cortex, the thalamorecipient cells which were identified through the monosynapticity of thalamic input established via pharmacological bath applications of magnesium and calcium (see Paper 2, Methods). These neurons also exhibited similar morphology and firing properties as the PT and IT neurons.

3.2.2 Dissecting the basic microcircuit bauplan of LPal/cortex

A significant part of the study focused on dissecting the synaptic input to the main output cells, the PT-type neurons. We examined three sources - thalamic input, intratelencephalic input and olfactory input (see Figure 7). The thalamic input reached the PT-type cells polysynaptically, via both excitatory and inhibitory intercalated cells. Cells which receive monosynaptic thalamic input were also characterised as mentioned earlier and were named thalamorecipient

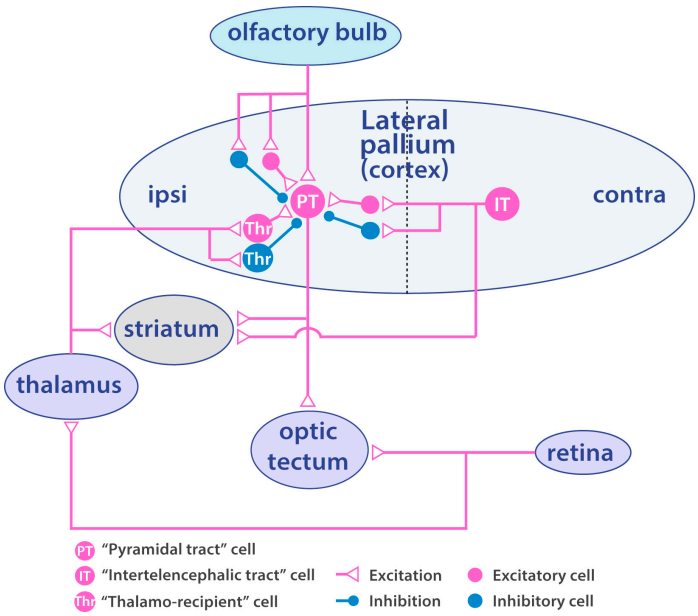

Figure 7. LPal microcircuit. Schematic showing synaptic input to the main output cells the PT-type cells. Thalamic input, including visual input is relayed via thalamorecipient cells to PT-type cells. Intratelencephalic input is also relayed polysynaptically. Olfactory input is relayed via the olfactory bulb.

cells (Figure 7). The thalamic input was glutamatergic and mediated by NMDA and AMPA receptors and the thalamic neurons projecting to LPal/cortex were located in the dorsal thalamus, some of which were immunopositive for calretinin (see Figure S5, Paper 2). While characterising thalamic input involved the general extracellular stimulation of the thalamic fiber bundle, more specifically, visual input was shown to be relayed via the thalamus through stimulation of the contralateral optic nerve which resulted in EPSPs and IPSPs in LPal cells (Figure 7, see also Figure S6, Paper 2). We showed subsequently (see Paper 3) that somatosensory input from both the body and head also reached the thalamus and was relayed to the LPal. Thus, the thalamus serves as an important relay of sensory inputs to the LPal/cortex in the lamprey as in mammals.

The second source of input to the LPal/cortex, intratelencephalic input from the contralateral hemisphere was also glutamatergic and mediated via both NMDA and AMPA receptors. It was also relayed polysynaptically via intercalated excitatory and inhibitory neurons to the PT-type neurons.

3.2.3 The primordial cortical microcircuit

The overall results from paper 2 showed that the LPal/cortex has a basic input-output circuitry which has been shown to be conserved from reptiles to mammals with layer 5a and 5b and layer 4 like neurons (Harris and Shepherd, 2015; Shepherd, 2011). The morphologies of the LPal excitatory neurons resemble their pyramidal neuron counterparts in that there are spiny and are layer spanning, extending to the outer molecular layer. While the typical pyramidal neuron has an apical dendrite and basal dendrites (DeFelipe and Farinas, 1992), the LPal neurons lack basal dendrites and this is probably compensated by the second layer spanning dendrite and the rich ramifications. The morphology of the LPal excitatory neurons are similar to the morphologies seen in the pyramidal neurons of the dorsal and lateral cortici of reptiles which also typically have two main dendrites (see Literature review) (Ulinski, 1990). The classical firing patterns seen in the mammalian cortex, the regular and burst firing are also typical of LPal neurons (McCormick et al., 1985). The excitatory neurons are located in the outer molecular layer while the GABAergic neurons are concentrated more in the inner molecular layer which is similar to the organisation seen in the three-layered dorsal cortex of reptiles (Ulinski, 1986; Ulinski, 1990). Furthermore, the PT-type neurons in the LPal, the reptilian dorsal cortex and the mammalian neocortex receive thalamic input polysynaptically (Shepherd, 2011). The layer 4 neurons in the mammalian neocortex receive monosynaptic input which is similar to thalamorecipient neurons in the LPal/cortex. In summary, significant similarities can be found in the microcircuit, afferent sensory inputs, morphology and physiology of constituent cell types in the lamprey LPal/cortex, the reptilian dorsal cortex and the mammalian cortex suggesting a common ancestry of these features.

3.3 PAPER 3: SENSORY REPRESENTATION IN THE LPAL

In the third paper (Suryanarayana et al., under review), we examined the organisation and representation of sensory modalities in the LPal/cortex. The LPal had been thought of as a primarily olfactory structure (Northcutt and Wicht, 1997). Our focus here was on visual and somatosensory inputs.

3.3.1 Delineating the visual area

To examine if the lamprey had a visual representation in LPal, we used an *in vitro* eye-brain preparation, which allowed us to record from the LPal while stimulating different parts of the retina extracellularly (Figure 8A). The stimulations of the retina elicited LFP responses in a circumscribed part in the dorsal part of the LPal/cortex - the *visual cortex*. Furthermore, when specific parts of the retina were stimulated, responses were recorded from only specific areas within the visual area (Figure 8B & 8C). This demonstrated a retinotopic organisation of the visual input as shown in the heatmaps of the retina where the yellow areas represent the area

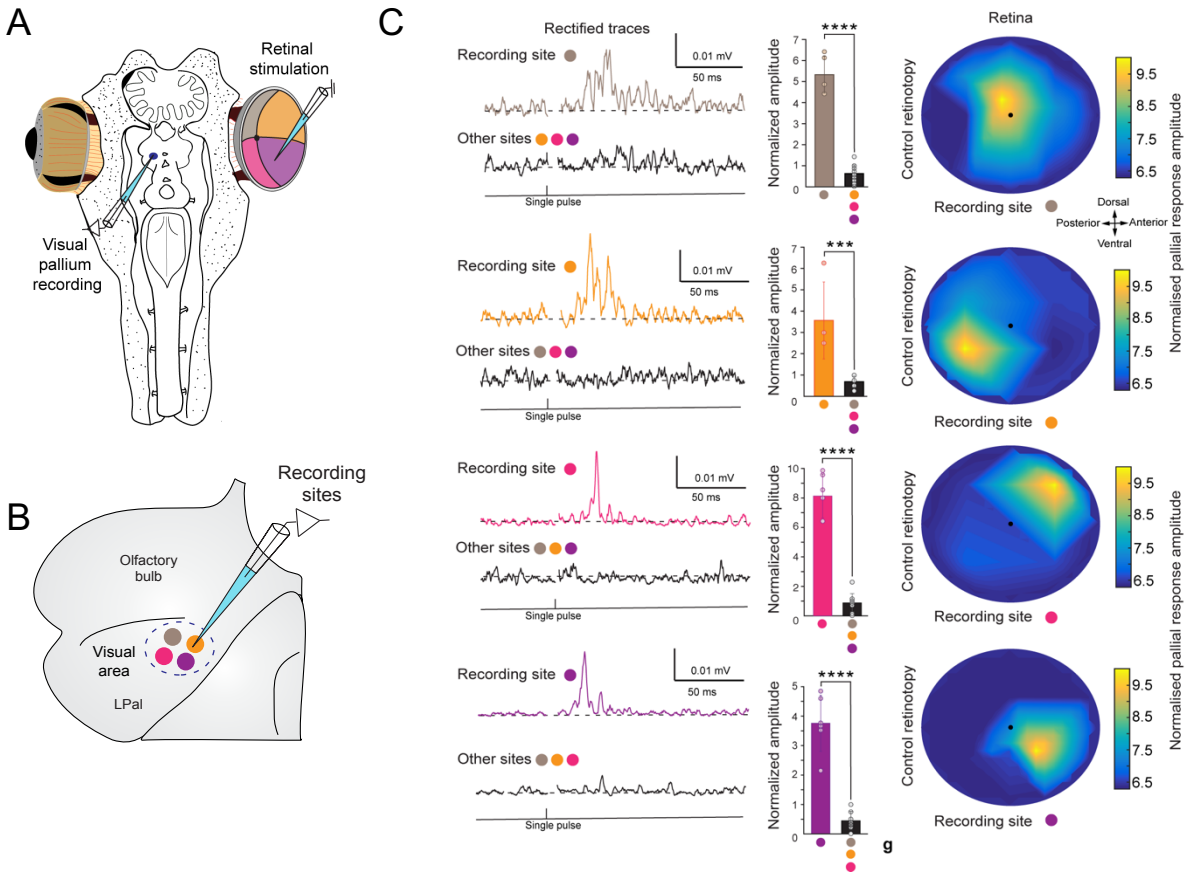

Figure 8. Retinotopic visual cortex A. Schematic of the eye-brain preparation used for extracellular recordings from LPal, while stimulating different parts of the retina **B.** Dorsal schematic of pallium showing the visual area and the location of the recording sites **C.** Responses in the visual area with retinotopic specificity with heat maps showing the areas in the retina which elicited responses in the colour-coded recording sites.

of the retina represented in a specific recording area within the visual cortex. In addition, local injections of gabazine in the visual cortex resulted in a long-lasting and increased activity and a loss of retinotopic specificity. This indicated that visual input activates both excitatory and inhibitory neurons in the visual cortex and in addition, the inhibitory neurons have a role in maintaining the topography. Furthermore, we verified that visual stimuli presented on a computer screen indeed conveyed information to the visual cortex, by using a similar *in vitro* eye-brain preparation but with the eyes intact. The stimuli consisted of a black point expanding in size – a looming stimulus as well as alternating black and white screens. Both elicited field potential responses in the visual area. We furthermore recorded intracellularly from the visual cortical neurons and could drive the neurons to spiking upon stimulation of the optic nerve/chiasm. This established that the LFP activity recorded during extracellular recordings were indeed population activation of visual cortical neurons.

3.3.2 Delineating the somatosensory area

We used a similar eye-brain preparation for mapping somatosensory areas in the LPal/cortex (Figure 9A). Extracellular stimulation of the dorsal column which relays sensory information from the body, and the trigeminal nerve which conveys sensory input from the head, revealed LFP responses in a distinct somatosensory area in LPal/cortex (Figure 9B). Furthermore, the responses elicited from dorsal column and trigeminal nerve stimulation were represented in distinct areas within the somatosensory cortex. This means that the somatosensory representation is somatotopic - in that representation from the head and body is distinct within the somatosensory cortex. The somatosensory area was located more lateral and caudal to the visual area and was distinct also from the motor area (Figure 9C).

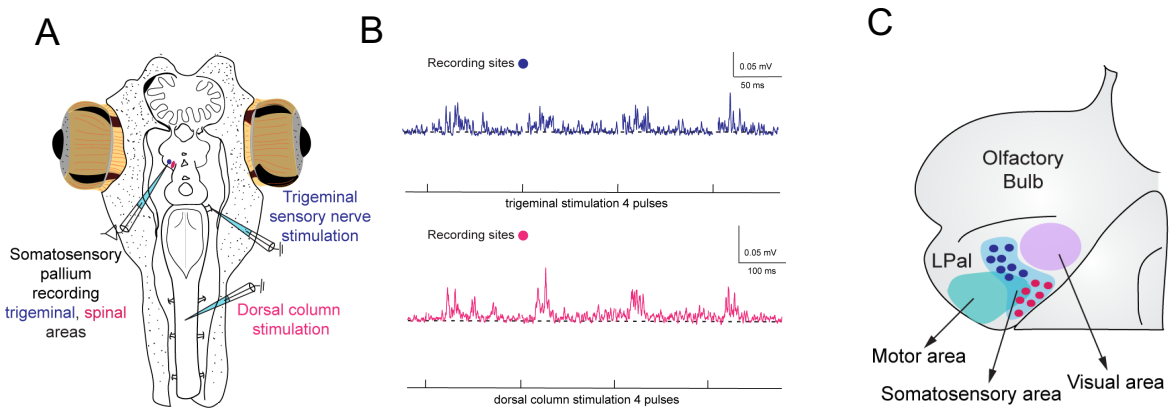

Figure 9. Somatosensory cortex A. Schematic of the preparation used to record extracellularly from LPal while stimulating the dorsal column and the trigeminal sensory nerve **B.** Responses in the somatosensory area in response to trigeminal sensory nerve and dorsal column stimulation. **C.** Schematic of a dorsal view of pallium showing the somatosensory area in relation to the visual area. The blue and pink dots represent responses elicited from the trigeminal sensory nerve and the dorsal column, respectively.

3.3.3 Thalamus as a major sensory hub

An important aspect of sensory cortical processing is the thalamic relay of sensory information. We investigated the thalamic relay of both visual and somatosensory input to LPal/cortex. We prelabelled thalamocortical neurons by dextran injections in the LPal for electrophysiological recordings. Some of the thalamic neurons send their dendrites into the optic tract where they

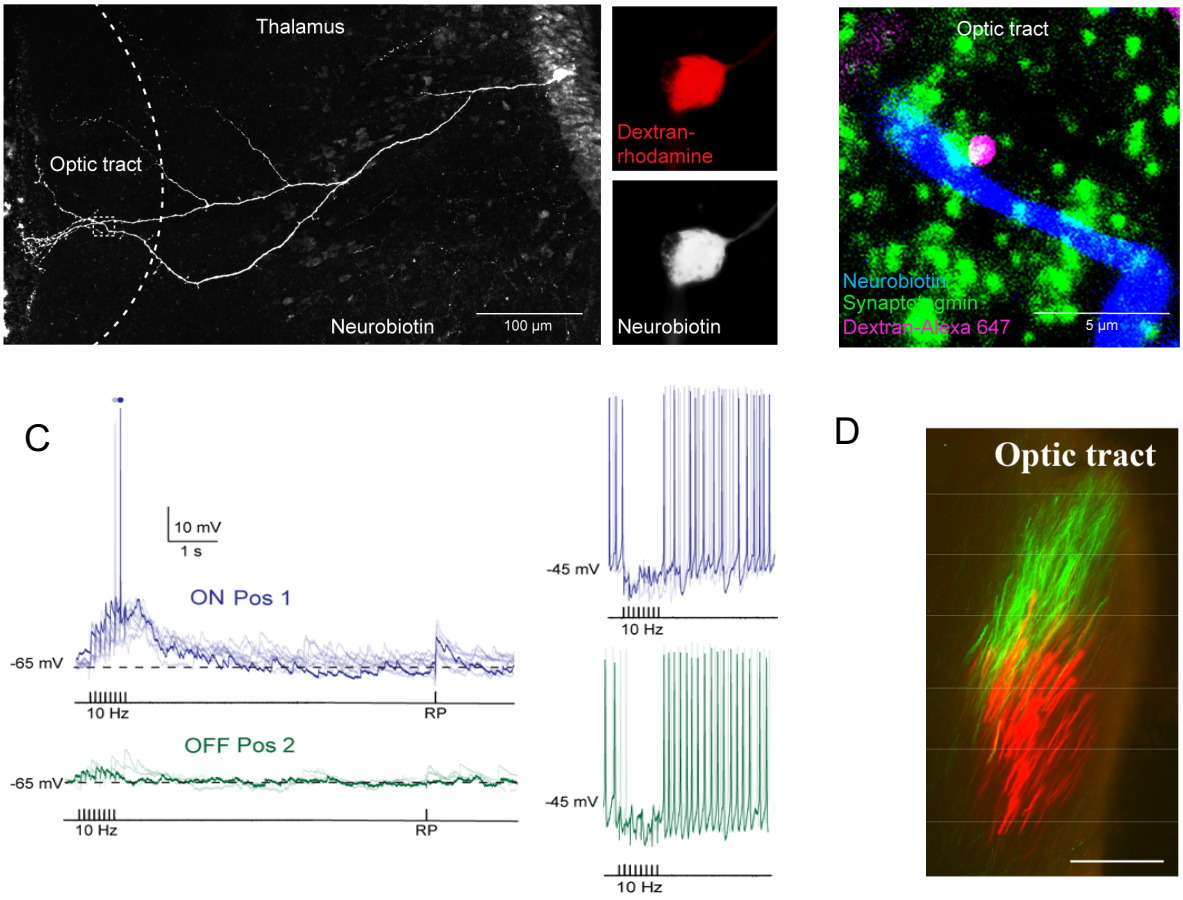

Figure 10. Retinotopy in thalamus A. A thalamopallial cell sensing dendrites into the optic tract **B.** The dendrite of the thalamopallial cell receives input from the optic tract **C.** A given thalamopallial cell responds to the stimulation of one part of the optic tract. **D.** Retinotopic organisation of optic tract fibres from (Jones et al., 2009).

receive visual input (Figures 10A and 11C). Furthermore, these neurons respond to extracellular stimulations of only one part of the optic tract and given that the fibres in the tract have been shown to be organised with retinotopic specificity (Figure 10D; (Jones et al., 2009), it indicates that the thalamic neurons also maintain this specificity. This furthermore implies that retinotopy is a feature inherent at all three levels of visual processing in the lamprey brain (Figure 11A) - the visual cortex, thalamus and as shown earlier, the optic tectum (homologue of the superior colliculus) (Jones et al., 2009). We also showed that fibres from the dorsal column nucleus (DCN) terminate in the thalamus and thalamocortical cells receive input from DCN fibres (Figure 11D). In the lamprey, it was known earlier that the DCN receives input

from the dorsal column, but it was unclear whether the DCN projected to thalamus (Dubuc et al., 1993). Furthermore, we showed that DCN neurons retrogradely labelled from

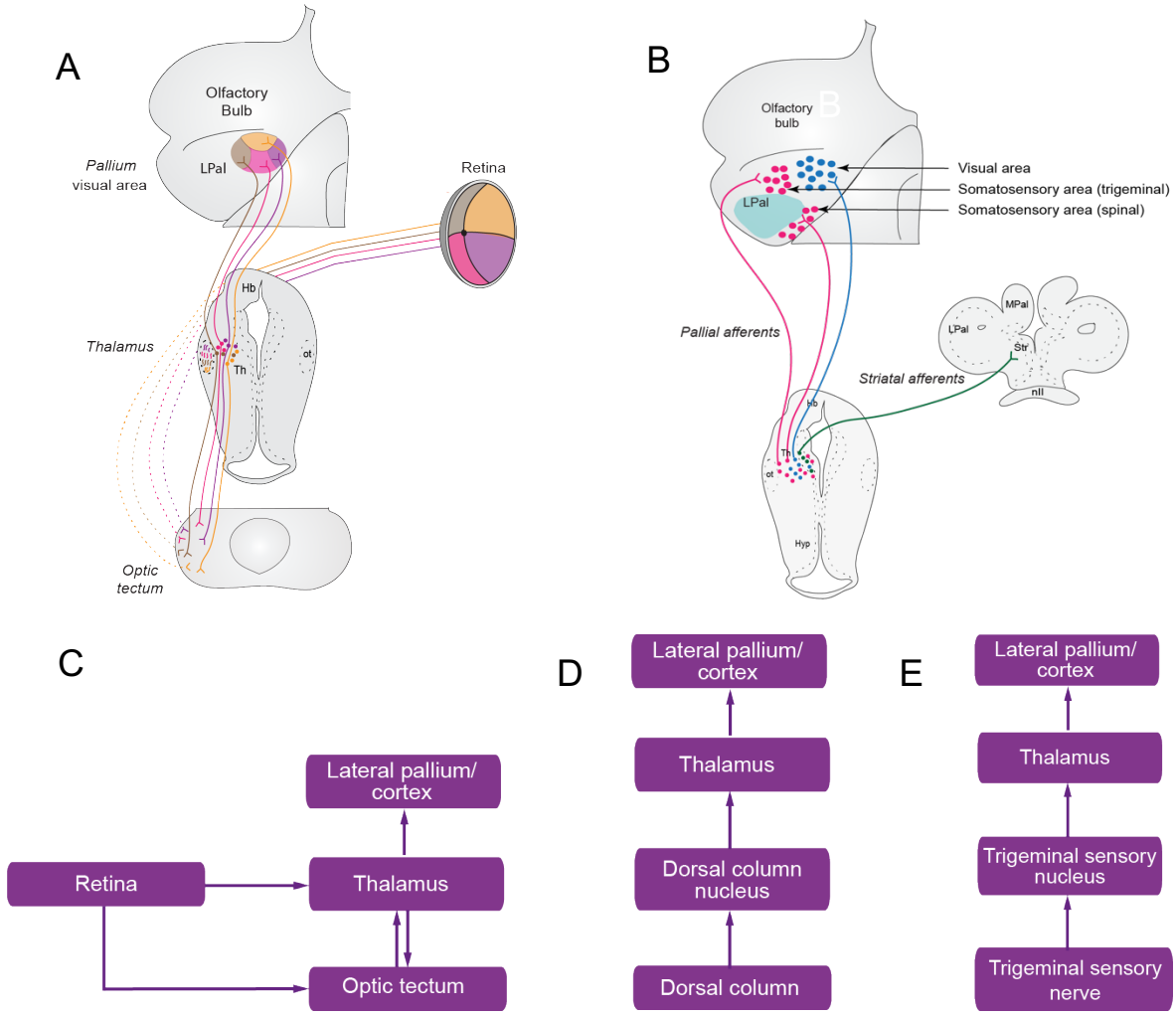

Figure 11. Thalamus A. Retinotopy maintained at three levels of visual processing in the lamprey brain, the optic tectum, thalamus and LPal. **B.** Thalamic neurons projecting to the visual and somatosensory cortex, as well as the striatum are distinct subpopulations. **C.** Input from the retina is relayed via the thalamus and a secondary pathway of processed information from the optic tectum also relayed via the thalamus to LPal. **D.** Input from the dorsal column reaches the dorsal column nucleus, which relays information to the thalamus. **E.** Trigeminal input is relayed via the trigeminal sensory nucleus to the thalamus. The thalamus in turn relays both these streams of somatosensory information to the somatosensory cortex.

the thalamus receives input from anterogradely labelled dorsal column fibres (Figure 10B), verifying that somatosensory information is relayed via the DCN to thalamus, in turn relays to LPal/cortex. Similarly, the trigeminal input is relayed via a trigeminal sensory nucleus, which has been referred to as the *nucleus of the radix descendens nervi trigemini* (Nieuwenhuys, 1997) that projects to the thalamus (Figure 11E). The thalamic neurons projecting to visual and somatosensory areas in the LPal/cortex as well as those projecting to the striatum (Ericsson et al., 2013a) were shown to represent different subpopulations (Figure 11B). The thalamic

neurons projecting to the striatum are located in the periventricular part of thalamus, while those projecting to LPal/cortex are located in both the periventricular and lateral parts (Figure 11B). The thalamus thus behaves as a major relay of sensory information and is of critical importance for cortical sensory processing in the lamprey, as in mammals.

3.3.4 Sensorimotor representation in LPal

Taken together, the results of paper 3 showed the presence of distinct visual and somatosensory areas, in addition to the motor area (Paper 1) in the lamprey LPal/cortex (Figure 12). These areas were located in the dorsal parts of the LPal, which has been thought to be the dorsal pallial homologue (Puelles et al., 2019). The visual area is retinotopically organised and the

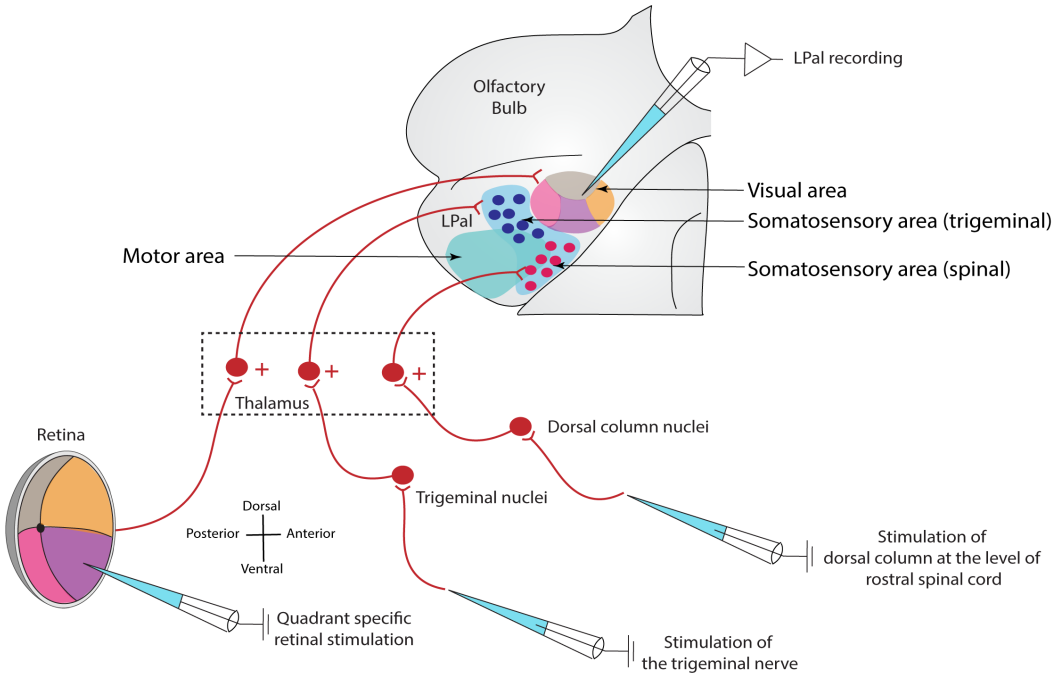

Figure 12. Sensorimotor areas in LPal/cortex. Schematic of the pallium showing the different sensory and motor areas along with the sensory relay pathways via the thalamus.

somatosensory input is organised with a basic somatotopy. Distinct sensory and motor areas are a hallmark of neocortical organisation in mammals and has been demonstrated in all mammals examined (Briscoe and Ragsdale, 2019; Kaas, 2013; Raghanti, 2017). There are also distinct representations of vision and somatosensation in the dorsal cortex of reptiles and in the avian Wulst (Briscoe and Ragsdale, 2019; Dugas-Ford and Ragsdale, 2015) see also Literature Review). The presence of a similar organisation in mammals, reptiles and lamprey gives rise to the compelling possibility of this being an ancestral and conserved aspect of dorsal pallial organisation across vertebrates. This also overturns the long-held notion that the anamniote pallia are largely olfactory (Karamian et al., 1966; Northcutt, 2011; Northcutt and Wicht, 1997; Wicht and Northcutt, 1998). Furthermore, the sensory streams of somatosensory information, both trigeminal and spinal are relayed via the trigeminal sensory nucleus and the DCN to thalamus. Retinal afferents target the thalamus directly as shown earlier (Heier, 1948; Kennedy

and Rubinson, 1977) but also indirectly via the optic tectum. Thus, the thalamus is an important relay of multi-modal sensory input to the cortex in lamprey as in other vertebrates. The thalamic neurons projecting to various sensory areas in the lamprey cortex are distinct, while in mammals they form distinct sensory relay nuclei (Sherman and Guillery, 1996).

3.4 PAPER 4: CONNECTIVITY OF THE SNC

In this study we examined the afferent and efferent connectivity of the subcortical dopaminergic substantia nigra *pars compacta* (SNc). The dopamine system is a vital aspect of motor control, reward system as well as in the detection of novelty in sensory processing across vertebrates (Bromberg-Martin et al., 2010; Redgrave and Gurney, 2006; Schultz et al., 1997; Yamamoto and Vernier, 2011). The SNc is an important part of the basal ganglia in all vertebrates and the basal ganglia in lamprey is highly conserved (Grillner and Robertson, 2016; Stephenson-Jones et al., 2011).

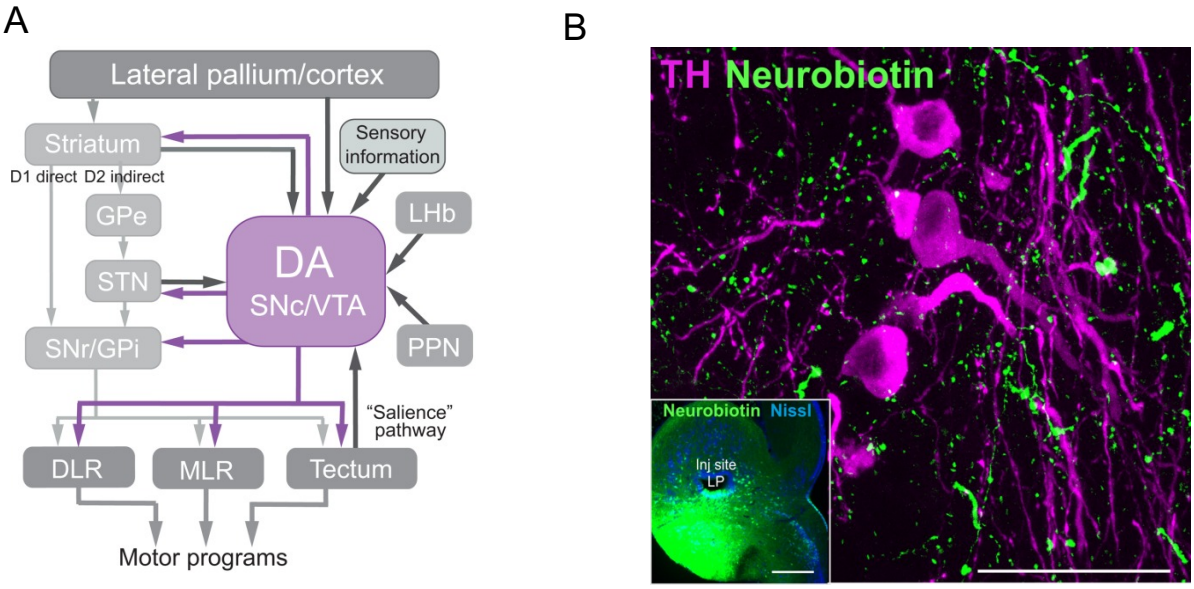

Figure 13. Conserved connectivity of the SNc in lamprey: A. Schematic showing the afferent and efferent connectivity of the dopaminergic SNc in the lamprey. **B.** Photomicrograph (right) showing the injection site in LPal and a confocal image of LPal efferents in close apposition to the dopamine neurons of the SNc.

The nucleus of the posterior tuberculum (NTP), located in the caudal diencephalon, has been considered the lamprey homologue of the mammalian SNc (Pombal et al., 1997; Stephenson-Jones et al., 2011). We examined the connectivity of the NTP through anterograde and retrograde tract tracing. The results showed that the dopaminergic efferents targeted the basal ganglia subnuclei, including the striatum, subthalamic nucleus and the SNr/GPi as in other vertebrates. The overall connectivity provides additional evidence supporting the homology of the lamprey NTP with the mammalian SNc. With regard to the projections to striatum in mammals, there are two dopamine pathways: one targeting the dorsolateral striatum as the nigrostriatal pathway and is implicated in motor functions (Graybriel 2008). The lamprey dopamine pathway is more akin to this pathway given that the lamprey striatum has similarities

with the dorsal striatum of mammals (Stephenson-Jones et al., 2011). The other dopamine pathway in mammals is from the ventral tegmental area (VTA) called the mesolimbic pathway that targets the ventromedial striatum. Whether this pathway exists in the lamprey is not clear. Furthermore, the dopamine input also reaches the cortex in mammals as the mesocortical pathway and we have evidence from tracing studies that TH-positive SNc neurons reaches LPal/cortex (unpublished data). Thus, this pathway also appears to be present in the lamprey.

Other than the projections to the telencephalon, there were also dopaminergic projections to the downstream motor centres including the DLR, MLR and the optic tectum (homologue of the superior colliculus).The SNc also has virtually identical input as in mammals, which originate from the lateral habenula, the pedunculopontine nucleus (PPN) and the optic tectum.

A major source of input to the SNc is from the LPal/cortex, which monosynaptically targets the dopaminergic neurons (Figure 13B). It has been shown that cortical projections to the SNc/VTA in mammals has a modulatory effect on dopamine neuron activity. For instance, sparse projections have been reported from the prefrontal cortices to the SNc/VTA in primates and more dense projections have been reported from homologue cortical areas in the rodent (Frankle et al., 2006; Takagishi and Chiba, 1991). Much less is known about pallial projections to the SNc homologues in other anamniotes. In summary, the overall connectivity of the SNc (input and output) was thus highly conserved as shown in the Figure 13A.

In addition to the investigations of the connectivity of the SNc, the study also included the examination of the dopamine D2 receptor in the lamprey brain. The lamprey striatum had been shown to have both the D1 and D2 receptors expressed in two distinct populations of striatal spiny projection neurons suggesting that the dopamine system is highly evolved and conserved in the lamprey (Ericsson et al., 2011; Grillner and Robertson, 2016; Robertson et al., 2012). The expression pattern of the D2 receptor throughout the lamprey CNS was less well known and was addressed in this study in conjunction with the connectivity of the SNc. The results show that the D2 expression pattern in the lamprey CNS closely resembles the expression pattern seen in mammals.

4 CONCLUSIONS AND GENERAL DISCUSSION

My doctoral studies have focussed on the lamprey lateral pallium (LPal), an area considered to correspond to cortex in mammals. The physiology, cytoarchitecture and function of LPal was virtually unknown. The evidence from the four studies shows high degree of similarities of the LPal with the mammalian neocortex in terms of efferent connectivity, cytoarchitecture, sensorimotor organisation (with a distinct retinotopic visual area, a somatosensory area with somatotopy and a motor area), as well as function. This demonstrates the presence of a dorsal pallium in the lamprey and strongly suggests its common ancestry with the neocortex, thus pushing back the origins of the mammalian cortex to the dawn of vertebrate evolution.

4.1 DELINEATING THE LAMPREY CORTEX

The first study (Ocana et al., 2015) showed that LPal/cortex can generate different types of movements. Electrical microstimulation of circumscribed regions evoked well-delineated eye and orienting movements, locomotion and oral movements. Furthermore, distinct glutamatergic and monosynaptic projection neurons targeted all major downstream motor centres in the midbrain and brainstem, as well as a few projections extending even to the rostral spinal cord. The motor area was located in the caudolateral portion of LPal/cortex. The projection neurons targeting different downstream motor centres were distinct subpopulations. Essentially, the LPal was capable of generating different types of movements and had an efferent projection pattern essentially mirroring the motor areas of the mammalian cortex.

Following the characterisation of the efferent connectivity and a motor area, in the subsequent study (Suryanarayana et al., 2017), we examined the cytoarchitecture and physiology of constituent cell types. The LPal was revealed to have a three-layered laminated cytoarchitecture with a molecular layer and an outer and inner cellular layer - a three-layered cortex. GABAergic interneurons represented 22% of the total number of cells. Pyramidal tract-like (PT-type, layer 5b-equivalent), intratelencephalic (IT-type, layer 5a-equivalent) and thalamorecipient cells (layer 4-equivalent) were located in the cellular layer and extended their spiny dendrites towards the molecular layer. The physiological properties of these cell types were examined and found to be similar to those of their cortical/mammalian counterparts. Thalamic input was relayed polysynaptically to PT-type cells. The LPal thus was a three-layered cortex, and had an overall basic microcircuit organisation, similar to the three-layered reptilian cortex, three-layered mammalian cortex, as well as the neocortex.

While the efferent connectivity and microcircuit organisation suggested a common ancestry, the sensory organisation in the LPal/cortex was unclear. The lamprey pallium as most anamniote pallia, had largely been thought to be olfactory (Northcutt and Wicht, 1997). However, in Paper 3 (Suryanarayana et al., under review), we showed via extracellular field potential recordings that primary visual input, relayed via thalamus, was represented in a distinct region in the dorsomedial LPal/cortex in a retinotopic fashion. Whole-cell recordings

of LPal neurons during optic nerve stimulation revealed EPSPs followed by inhibition. Local gabazine injections in the visual area resulted in a large response, with a loss of retinotopy, indicating that GABAergic neurons were important for maintaining the specificity. Furthermore, somatosensory information from the DCN was also relayed via thalamus and represented in an area between the visual and motor areas (see Figure 12). Stimulation of the spinal dorsal column and trigeminal nerve elicited multi-unit responses in adjacent areas - a basic somatotopy, in that information from the face and body were represented in distinct regions in the somatosensory areas. Both visual and somatosensory information were relayed via distinct subpopulations of thalamocortical neurons with retinotopy present also at the level of thalamus. These results showed an unforeseen level of similarity with the sensorimotor organisation of the mammalian neocortex, as well as the sensory thalamocortical system.

4.2 SIMILARITIES SUGGEST CONSERVATION

When evaluating homologous structures, similarities signify an important evolutionary principle - *conservation and common ancestry*. In our three main studies focused on the LPal/cortex, we have looked at the connectivity, cytoarchitecture and sensorimotor organisation of the cortical homologue structure in the lamprey and compared it with that of mammals and as detailed above, it shows a remarkable degree of similarity. But the cortex in itself is not a stand-alone structure - the subcortical infrastructure is equally important and indeed, the basal ganglia in terms of organisation, connectivity, neurotransmitters, peptides and ion channel expression is conserved in remarkable detail. Furthermore, the habenula, the dopamine system and the related forebrain structures also show a high degree of conservation (Ericsson et al., 2011; Ericsson et al., 2013b; Grillner and Robertson, 2016; Perez-Fernandez et al., 2014; Stephenson-Jones et al., 2012; Stephenson-Jones et al., 2013; Stephenson-Jones et al., 2011). An important aspect of the connectivity of the subcortical dopaminergic SNc in paper 4 of this thesis, shows that the afferent and efferent connectivity of the SNc is virtually identical with that of mammals.

One should also consider in this context, the significant similarities in the early patterning of the embryonic telencephalon in general and pallium in particular, of the lamprey with that of other vertebrates (Sugahara et al., 2016, 2017). When considered together, the data strongly supports our interpretation that important aspects of the vertebrate forebrain design had evolved very early in vertebrate evolution and have been maintained notwithstanding the great variety of forebrain structures, in particular the pallium, seen across vertebrates (Briscoe and Ragsdale, 2019). We conclude that this high degree of similarity is unlikely to have evolved through convergence, but rather can be explained by common ancestry.

4.3 IMPORTANCE OF FUNCTION IN EVOLUTIONARY COMPARISIONS

We would like to emphasise the importance of considering function while evaluating evolutionary relationships. Natural selection acts on the behavioural output of circuits -

function. Function importantly shapes homologues, and a fundamental principle of evolution in simplistic terms is - *if something works, then retain it and build on it*, as evidenced in the case of the dorsal pallium presented in this thesis, but also in the well-conserved basal ganglia (Grillner and Robertson, 2016), notwithstanding the unusual plasticity observed in the evolution of the forebrain (Briscoe and Ragsdale, 2019). This in our view, reflects the extraordinary ability of evolution to adapt to the significant variability and requirements in sensorimotor processing and behaviour across species. It has been contended that function is irrelevant while defining homologies and that it is epiphenomenal relative to brain structure. Our evidence clearly contravenes this argument - and we would like to reiterate that our data suggests that it is inconceivable that function is, in its entirety, epiphenomenal. Brain structures have evolved with functional constraints and not the other way around.

4.4 POSSIBLE EVOLUTIONARY SCENARIO OF VERTEBRATE CORTICI

Our data allows us to propose a possible scenario of the evolution of laminated cortici across vertebrates. We detail this in three sections below.

4.4.1 Cell types

A major aspect of the evolution of the neocortex is the evolution of cell types. The major output cells of the neocortex, the layer 5b (PT-type) cells, that target the brainstem and spinal cord seem to have been conserved in terms of their projection pattern and function. They are the cells relaying the cortical command to downstream motor centres. These projections arise from sensory and motor areas of the neocortex and they also do so in the dorsal pallial homologues in sauropsids (See Literature Review), as well as lamprey. In addition, the IT-type cells also appear to be conserved in terms of their projection pattern. Moreover, the thalamorecipient cells are a conserved subtype. Thus, the three basic cell types - *Output, Input and IT cells* are found in proposed dorsal pallial homologues in the lamprey, modern sauropsids (in the dorsal cortex of non-avian reptiles and the Wulst in avians) and mammals. Furthermore, Briscoe and Ragsdale (2018a) propose that the ancestral reptile dorsal pallium had these three cell types; and the three-layered dorsal cortex and its possible modification into the Wulst arising as an independent evolution seen in sauropsids. One could thus propose that the common sauropsid ancestor probably possessed a three-layered cortex with these three cell-types (Figure 14, modified from Briscoe and Ragsdale., 2018) and that this is a conserved feature of the dorsal pallium. And indeed, our own results showing a three-layered cortex similar to that of the reptilian dorsal cortex supports this view. It would appear that evolution has retained the connectivity aspect of these cell-types and there has been a conspicuous increase in the number of these cells during evolution and a dramatic generation of new excitatory cell types in mammals in terms of their molecular identity (Tosches and Laurent, 2019). Furthermore, these cells appear to differ in their marker expression between different areas of the neocortex - for instance between the visual cortex and the premotor cortex (Tasic et al., 2018). It is conceivable

that these new molecularly defined excitatory cell types have emerged from ancestral ones and this seems to be a fundamental principle of neocortical evolution.

4.4.2 Microcircuit and Lamination

The neocortex with its six-layered cytoarchitecture emerged with the last common ancestor of mammals (Kaas, 2013). The homologue region of the mammalian neocortex in reptiles is the anterior dorsal cortex (Tosches et al., 2018), a three-layered structure. It must be noted that also in mammals other parts of the cortex, in particular the olfactory piriform cortex and the hippocampus are three-layered (Klingler, 2017), and in non-avian reptiles, so are the homologue regions the lateral pallium and the medial pallium (Literature review, Laurent et al. (2016b); (Naumann et al., 2015). Laminated cortices in pallial areas of anamniotes have not been investigated in significant detail but our results from the lamprey cortex being three-layered suggest that a simple three-layered cortex existed in the common vertebrate ancestor and that lamination has been another vital principle of cortical evolution. While there are pallial structures, which are non-laminated like the DVR in sauropsids, lamination seems to be an important cytoarchitectural feature. The six-layered neocortex could thus have evolved from the elaboration of the ancestral three-layered cortex, particularly with the addition of layer 2-3.

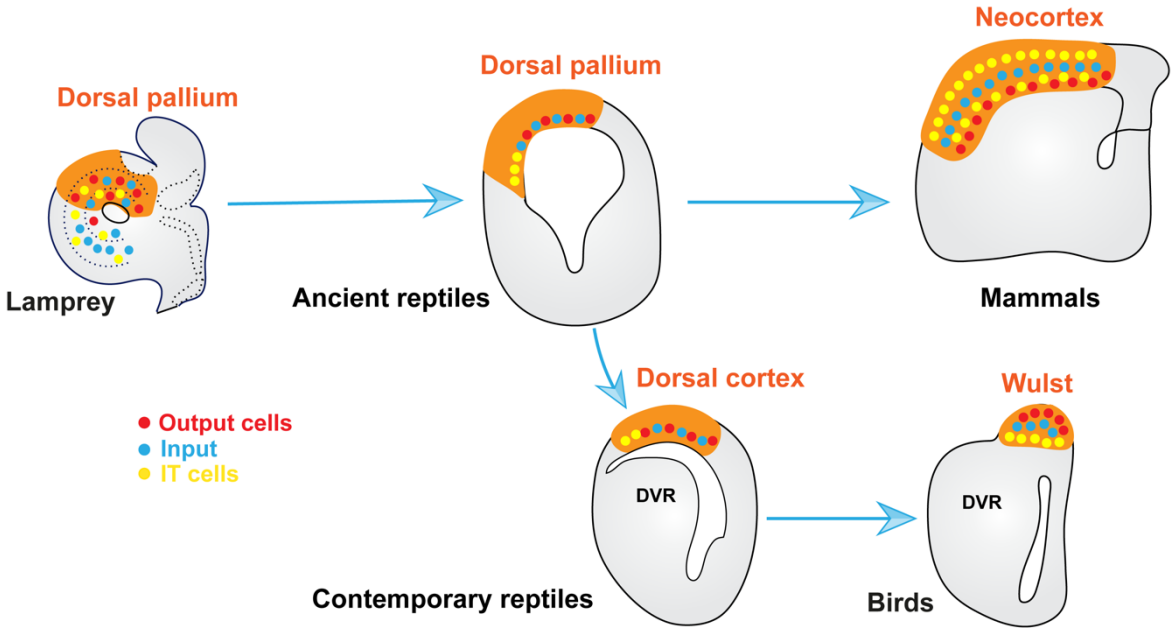

Figure 14. Proposed evolutionary scenario for the dorsal pallium. Schematic of the pallium showing the dorsal pallial homologues with basic input-output and intratelencephalic cell types proposed in the lamprey (cyclostomes), common reptilian ancestor and in modern day reptiles and mammals, modified from Briscoe and Ragsdale (2018a).

4.4.3 Modality specificity

Another principle of neocortical evolution is modality specificity (Figure 15). It is clear from our results that discrete areas of the dorsal pallium for processing distinct sensory modalities arose as a common ancestral plan and has been maintained during evolution. The modalities represented have been guided by species specific requirements. Furthermore, specificity of sensory processing of particular modalities like retinotopic mapping of the visual space or the distinct representations of the somatosensory inputs have also been maintained during evolution. This indicates that different species have a need to respond to similar external stimuli and reminds us once more, that during evolution, a mechanism which works well, is maintained. Furthermore, the relay of sensory information from the thalamus yet again in modality specific channels is also a well-conserved aspect of the dorsal pallium (Briscoe and Ragsdale, 2018a; Dugas-Ford and Ragsdale, 2015). Lastly, another of the major features of the neocortex are the associational areas, which process multimodal sensory inputs (Briscoe and Ragsdale, 2019). The ancestry of associational areas as yet, remains unclear. We do not know whether distinct associational areas exist in the lamprey LPal/cortex and the same is true for most non-mammalian species.

4.5 GOING BEYOND AMNIOTES

The majority of investigations of cortical homologues had been limited to amniotes and in the vast anamniote lineage, data available had been scarce. The evolutionary ancestry of the mammalian neocortex has remained unclear largely due to lack of significant data in anamniote species (Dugas-Ford and Ragsdale, 2015; Tosches and Laurent, 2019). The focus has been on generic developmental patterning of the embryonic telencephalon traced back to cyclostomes (Puelles, 2017; Sugahara et al., 2017), cell type specificity based on marker expression (Dugas-Ford et al., 2012; Tosches et al., 2018), as well as conserved microcircuit organisation (Harris and Shepherd, 2015; Karten, 2015; Shepherd, 2011). The consensus up till now had centred on a reptilian ancestry of the neocortex with the three-layered dorsal cortex proposed as the ancestral homologue (Briscoe and Ragsdale, 2018a; Tosches et al., 2018). Our studies are amongst the few examples of comprehensive and detailed examinations of cortical homologue structures in anamniotes. There is clearly a need for more investigations of pallia in the vast anamniote lineage - this will let us better understand and segregate ancestry from novelty.

4.6 TOWARDS A PAN-VERTEBRATE SCHEMA FOR NEOCORTICAL EVOLUTION

We propose based on the data presented above that the lamprey cortex represents several features of the pan-vertebrate ancestral condition, which has been maintained through evolution in basal teleosts, elasmobranchs (Ebbesson and Schroeder, 1971), amphibians and basal reptiles (Briscoe and Ragsdale, 2018a, 2019) to mammals. The mammalian neocortex has expanded greatly in size and also in lamination with the addition of layer 2-3 and finally the conspicuous expansion of the frontal areas in humans (Kaas, 2013; Northcutt and Kaas,

1995; Raghanti, 2017). The features present in the lamprey LPal/cortex are listed in Figure 15. We propose that a dorsal pallium was already present in the last common ancestor of all vertebrates with many of the features seen in the lamprey LPal/cortex – a three-layered cortex with functional cell types (Figure 14) and distinct visual (with retinotopy), somatosensory (with somatotopy) and motor areas (Figure 15).

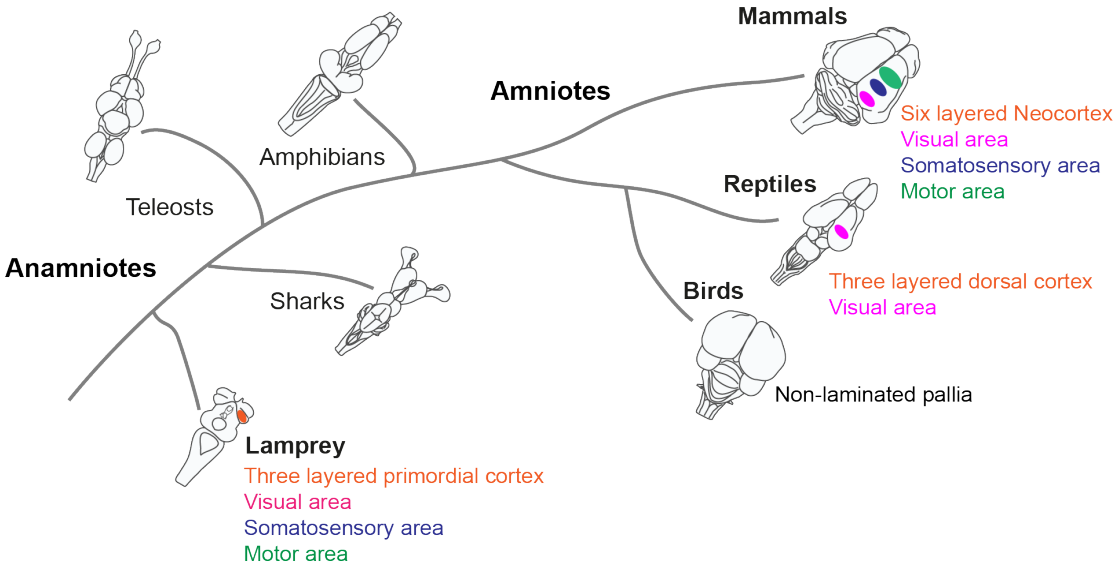

Figure 15. Modality specificity in dorsal pallial homologues across vertebrates. Schematic of the vertebrate evolutionary tress showing present knowledge of sensorimotor representations in dorsal pallial homologues in lamprey, reptiles and mammals.

There has been unusual plasticity in the evolution of the dorsal pallia across vertebrates. For instance, there is some data showing loss of certain neocortical features - lack of retinotopic specificity in the dorsal cortex of turtles or the lack of a proper laminated cortex in avians (it should be noted however that the Wulst does show lamination) (Fournier et al., 2018; Maler, 2018). These could be secondary losses acquired independently during evolution. There are still significant similarities in the dorsal cortex from a connectomic and molecular perspective in non-avian reptiles (Shepherd, 2011; Shepherd and Rowe, 2017; Tosches and Laurent, 2019; Tosches et al., 2018) and from a connectomic perspective in the DVR and Wulst of avians (Briscoe et al., 2018; Briscoe and Ragsdale, 2018a, b; Karten, 2015) with the neocortex. This, when considered together with our data from the lamprey LPal/cortex, which shows an even higher degree of similarities and function and alignment with the mammalian condition, strongly suggest that the dorsal pallium existed in the last common ancestor of all vertebrates with several features seen in the mammalian neocortex.

The work presented in this thesis has thus fundamentally altered our understanding of the lamprey pallium in particular, and the evolutionary ancestry and origins of the mammalian cortex in general. Furthermore, as shown earlier, the basal ganglia, the dopamine system and the habenula have been shown to be also conserved in remarkable detail (Grillner and Robertson, 2016). Taking together we come to the overarching conclusion that the important aspects of forebrain design underlying decision-making, and action selection are ancient and had evolved already at the dawn of vertebrate evolution.

5 FUTURE PERSPECTIVES

5.1 SENSORY PROCESSING IN THE LAMPREY CORTEX – MICROCIRCUITS TO BEHAVIOUR

While distinct sensory areas have been clearly described in the lamprey cortex, we know much less regarding neural mechanisms underlying sensory processing. One key element to consider for instance, is that even with a retinotopic organisation of the visual area, to what extent visual processing is similar to the mammalian visual cortex? What is the role of the different cell types in mediating this sensory processing and what type of visual input do these cells respond to? These are pertinent and interesting future avenues, which must be explored in relation to behaviour. One can also investigate a more detailed description of the basic somatotopy shown in the somatosensory areas - what is the nature of the *lamprunculus?*

The microcircuit of the lamprey cortex must also be detailed more in terms of recurring microcircuits, which are adaptable to different sensory and motor processing requirements. Neocortical circuits have been viewed as cortical columns (Hubel et al., 1977; Mountcastle et al., 1955; Mountcastle, 1957) based on the organisation of neurons in a column spanning all 6 layers. Another well-known scheme has been with the idea of canonical cortical microcircuits, which proposes recurring microcircuits encompassing the basic connectivity between two main modules – the excitatory cells and inhibitory cells spanning across layers (Bastos et al., 2012; Douglas and Martin, 2004). These schemes while useful, must be considered with their short- and long-range connectivity, the granularity of receptive fields of constituent neurons, in a behavioural context, plasticity and as more recent transcriptomic data have shown – the molecular identities of cell types. This needs to be done for different areas in order to understand the adaptability of any basic recurring microcircuit.

5.2 FROM THE PRIMORDIAL LAMPREY CORTEX TO THE NEOCORTEX

5.2.1 Using evolutionary conservation to reveal novelty

Given this inherent adaptability in cortical circuits, a powerful approach is to look at conserved cortical connectivity in homologue areas in a wide array of species at critical phylogenetic positions. This is vital to dissect out innovations from ancestry. Looking at relatively simpler homologue structures and the microcircuits controlling yet again, relatively simpler sensorimotor processing as with our lamprey results, will provide us with tantalising clues into conserved aspects of circuits mediating behaviour. When examined across different vertebrate species, we can furthermore, relate the expansion of cortical circuits vis-à-vis the extended sensorimotor repertoire of mammals.

5.2.2 Need for a functional definition of cell types

More recent transcriptomic work is providing data on different cell types in the neocortex on an unprecedented level of detail (Huang and Paul, 2019; Krienen et al., 2019; Tasic et al., 2018;

Yuste et al., 2019). This has been used also to explore conservation and novelty of cell types in the reptilian dorsal cortex (Tosches and Laurent, 2019; Tosches et al., 2018) and indeed, a similar classification of the cell types in the lamprey cortex would be an interesting next step. While the transcriptomic dataset provides one way of classification and also opportunities to study specific cell types, these must also be defined, most critically, in terms of *function* and their role in behaviour. Defining microcircuits mediating behaviour from a cell-type perspective is the exciting and inspiring future of neocortical research.

6 METHODOLOGICAL CONSIDERATIONS

The studies in the thesis use several techniques which can be subdivided into three main divisions – anatomical tract tracing, immunohistochemistry and electrophysiology. The detailed methodologies are described in individual papers; we will only consider briefly the merit of the techniques used and present a generic overview. Lampreys of either sex belonging to two species - *Lampetra fluviatilis* and *Petromyzon marinus* were used. The experiments were performed in conformance to the approved guidelines of the local ethics committee (*Stockholms Norra Djurförsöksetiska Nämnd*) and were in accordance with The Guide for the Care and Use of Laboratory Animals (1996). Every effort was made to minimize suffering and to reduce the number of animals used during the studies.

6.1 ANATOMICAL TRACT TRACING

To delineate neuronal connectivity, we used anterograde and retrograde transport of neuronal tracers, which included Neurobiotin and a host of fluorescent coupled dextran amines (3 kD and 10 kD). The transport of Neurobiotin was accomplished in *in vitro* experiments while the transport of dextran amines required experiments in which the animal was returned to their aquarium following injections for 48-72 hours to allow for the transport of the tracer. This procedure was also followed for retrogradely labelling neurons of interest for electrophysiological recordings.

We followed several caveats while interpreting tracing data:

- All tracing results were verified bidirectionally – with anterograde and retrograde tracing
- Injection sites were always analysed without exception to rule out possible spread of the tracers
- Traced projections were verified generally with electrophysiological recordings
- Close appositions and presumed synaptic contacts between labelled fibres were verified using synaptic markers and/or electrophysiology
- Traced projections and labelled populations were imaged using fluroscent microscopy while synaptic contacts were imaged and analysed using confocal microscopy

6.2 IMMUNOHISTOCHEMISTRY

Immunohistochemistry to identify neuronal subpopulations based on molecular marker expression were used. We used a variety of primary antibodies (monoclonal and polyclonal) raised against particular antigens. These included neurotransmitters (GABA), calcium-binding proteins (parvalbumin, calbindin, calretinin), neuropeptides (vasoactive intestinal peptide (VIP), neuropeptide Y (NPY), somatostatin, cholecystokinin (CCK)), tyrosine hydroxylase and the presynaptic marker synaptotagmin. These antibodies were applied to sections of varying thickness, which were incubated for 24 -72 hours. For visualisation of the target protein, the

sections were subsequently incubated with fluorescent secondary antibodies. The specificity of the antibodies was confirmed by checking through the results obtained by different antibodies raised against the same antigen in different species. The antibodies used had been tested for the specificity of the antigen recognition and we also performed Western blot to confirm specificity (See Suryanarayana et al. (2017) supplementary figures).

6.3 ELECTROPHYSIOLOGY

6.3.1 Intracellular recordings

For the preparation utilised to record from neurons in cortex/thalamus while extracellularly stimulating different areas, the angle of sectioning was adjusted in such a way that both the recording and the stimulation site could be visualised. A thick or thin slice (300 μm to 2mm) was mounted in a recording chamber continuously perfused with aCSF at 6-8°C and allowed to recover for one hour. Whole-cell patch clamp recordings were performed with microcapillaries. The resistance of recording pipettes was 7–12 MΩ when filled with an intracellular solution. In some cases, the electrode solution also included 2 mM of triethylammonium bromide (QX314) to block action potentials.

To retrogradely label neurons prior to the patch clamp recordings, rhodamine conjugated dextran amine was injected unilaterally into the target area. Following injections, the dorsal skin was sutured, and the animals returned to their aquarium for 48-72 hrs to allow for transport of the tracer. The animals were then sacrificed, and the brains removed and embedded in agar. Transverse slices of region of interest were cut on a vibrating microtome. The slice was continuously perfused with aCSF at 6-8°C. Neurons were visualised with DIC/infrared optics. Retrogradely labelled fluorescent cells were visualised using a mercury lamp. For detailed methodological descriptions related to intracellular recordings see Paper 2.

6.3.2 Extracellular recordings

To record extracellular activity in the cortex in response to visual or somatosensory stimulation, we used an *in situ* preparation exposing the brain and rostral segments of the spinal cord maintaining the eyes. The preparation was then pinned down in a cooling chamber perfused continuously with artificial cerebrospinal fluid (aCSF, 6–8°C). Local field potentials (LFPs) were recorded using tungsten microelectrodes (~1-5 MΩ), connected to a 4-channel amplifier. The stimulation intensity was generally set to minimal strength necessary for evoking LFPs. For visual stimulation via a screen the same preparation was used albeit with the eyes intact, pinned down in a transparent chamber facing the centre of a computer screen placed in a lateral position at ~30 cm distance. For electrical stimulation of the retina, dorsal column or trigeminal nerve, borosilicate glass microcapillaries connected to a stimulus isolation unit were used. Visual stimuli were generated using custom code in Matlab, using the Psychophysics Toolbox extension and coordinated with the electrophysiological acquisition software programmable

pulse generator. Experiments were carried out in darkness, and prior to each experiment the preparation was left to adapt to a white screen (used as the background for the applied stimuli), for at least 30 mins. For detailed methodological descriptions related to intracellular recordings see Paper 3.

7 APPENDIX – LITERATURE REVIEW

7.1 PALLIA IN ANAMNIOTES

7.1.1 Cyclostomes

7.1.1.1 Lamprey

The lamprey has a relatively tiny telencephalon and the pallium, located caudal to the olfactory bulb consists of an evaginated portion called the lateral pallium and a medial pallium (Northcutt). The evaginated lateral part consists of a cellular layer with no visible evidence of lamination around a ventricle and an outer layer largely devoid of neurons (Nieuwenhuys, 1997; Northcutt and Puzdrowski, 1988; Northcutt and Wicht, 1997; Pombal et al., 2009). Several authors had subdivided the lateral pallium into a dorsal, lateral and ventral pallium in addition to the medial pallium based on anatomical location (Nieuwenhuys, 1977, 1997; Northcutt, 1981; Pombal et al., 2011; Pombal and Puelles, 1999). These have been thought to correspond to the similar pallial divisions in amniotes. However, no physiological, functional or anatomical data existed except for the olfactory input relayed from the olfactory bulb to the lateral pallium (Northcutt and Puzdrowski, 1988). Furthermore, although thalamic input was known to reach the pallium, it was shown to reach the medial pallium and whether it reached the lateral pallium was unclear (Northcutt and Wicht, 1997; Polenova and Vesselkin, 1993). Thus, the homologies of lamprey pallial areas to aminiote pallial areas has remained controversial as is the question whether there is a dorsal pallium (neocortical homologue) in the lamprey.

More is known regarding homology from developmental data from the embryonic lamprey. The expression patterns of developmental regulatory genes show evidence of regionalization in the lamprey larval telencephalon. A segregation of pallial and subpallial regions has been shown, based on Pax6 expression in the dorsal portion of the telencephalon, which is the pallial portion, and Dlx1/6 expression in the ventral subpallial regions (reviewed in (Kuratani et al., 2002; Murakami and Kuratani, 2005; Murakami et al., 2002; Murakami and Watanabe, 2009). This distinction has also been recognised by the Drosophila distal-less protein expression (Martinez-de-la-Torre et al., 2011). Additionally, expression of Emx 1, which is a marker of the gnathostome dorsal, medial and lateral pallia, has been shown in the dorsalmost portion of pallium of late lamprey embryos and early prolarvae (Myojin et al., 2001) reviewed in (Kuratani et al., 2002). Thus, there is developmental evidence in the lamprey for the gnathostome neocortical, piriform and hippocampal homologues. Until recently, it was thought that lampreys lacked the medial ganglionic eminence (one of the subdivisions of the subpallium, which gives rise to cortical interneurons) due to the presumed absence of a Hedgehog- and Nkx2.1-positive subpallial domain (Sugahara et al., 2011), although GABAergic neurons are observed in the pallium of the juvenile and adult lamprey. In a study

from the same group, however, all major pallial domains including the dorsal and ventral pallium and both the lateral and medial ganglionic eminences have been identified in the lamprey as well as in the hagfish (Sugahara et al., 2016) and reviewed in (Sugahara et al., 2017).

7.1.1.2 Hagfish

The monophyletic cousin of the lamprey and the other extant cyclostome is the hagfish. Hagfishes are deep sea scavengers and have degenerated eyes although they possess a retina (Locket and Jørgensen, 1998; Wicht and Northcutt, 1998). The brains of the hagfish at the outset are quite dissimilar in relation to the lamprey and gnathostomes and this had given rise to doubts on the monophyly of lamprey and the hagfish. Recent fossil evidence and earlier molecular evidence, however, support the monophyly (Heimberg et al., 2010; Miyashita et al., 2019). The pallium in hagfish has been studied in terms of its overall anatomical organisation and is highly differentiated with a large laminated structure, with at least five alternating layers of cells and fibres (Ronan and Northcutt, 1998; Wicht and Northcutt, 1998). However, different pallial subdivisions, like the dorsal, lateral and ventral pallia are not clear. Furthermore, the telencephalon in the hagfish unlike the lamprey and other gnathastomes the telencephalon fuses anteriorly with the olfactory bulb and with a unusually large connection to the diencephalon (Northcutt and Ronan, 1992). The pallium receives extensive secondary olfactory input as well as thalamic input indicating that in addition to the olfactory input, non-olfactory modalities may also be relayed to the pallium (Wicht and Northcutt, 1998). The pallium also has extrinsic projections to the tectum, contralateral telencephalon, olfactory bulbs and much less degree to the mesencephalic tegmentum (Wicht and Northcutt, 1998).

7.1.2 Bony fishes

In species phylogenetically younger than lamprey, like the ray-finned fish, there are conflicting theories on cortical homologies (Ito and Yamamoto, 2009; Northcutt, 2011). Ray-finned and lobe-finned fishes (Bony fishes) diverged from the main vertebrate line of evolution over 430 million years ago (Nieuwenhuys, 1963). Molecular evidence indicates the presence of the pallial and subpallial divisions in the developing telencephalon and there is also evidence to indicate migration of GABAergic neurons to pallial territories (Ganz et al., 2014; Maruska et al., 2017). Based on a combination of histochemical and genetic markers, there is some evidence for the presence of a dorsal pallium homologous to neocortex (Ganz et al., 2014; Mueller et al., 2011), and similarities in circuitry to the mammalian neocortex has been found in with non-olfactory modalities (visual, acoustic, somatosensory) relayed via the preglomerular complex which is considered similar to the sensory thalamus of mammals (Wallach et al., 2018; Yamamoto et al., 2007; Yamamoto and Ito, 2005). There is a variety of pallial cytoarchitectures ranging from simple neuronal populations clustered around the ventricle with little migration to more complex circuits with several cell groups and intrinsic

and extrinsic connections in some teleosts (Braford Jr. and Northcutt, 1974; Giassi et al., 2012; Kawaguchi et al., 2019). Lamination has however not been found in the teleost pallia (Ito and Yamamoto, 2009). Extratelencephalic projections have also been reported in teleosts with the projections targeting the optic tectum and the mesencephalic tegmentum (Murakami et al., 1983). Investigation of pallial homologies is faced with another challenge in teleosts – in that their pallium undergoes evagination, making comparisons tricky (Northcutt, 2008) as evagination reverses the spatial arrangement of pallial neurons, which is in contrast to other vertebrates.

7.1.3 Cartilaginous fishes

On the other hand, cartilaginous fishes (sharks, distinct from teleosts) and other fleshy-finned fishes join the lamprey, reptiles and mammals in the way the telencephalon develops (invagination). Sharks also have efferent projections from the telencephalon targeting the thalamus and optic tectum, as well as the brainstem and rostral spinal cord (Ebbesson and Schroeder, 1971). Early pioneering work on the shark pallium by Sven Ebbesson (Cohen et al., 1973; Ebbesson, 1970, 1980a, b; Ebbesson and Schroeder, 1971) overturned the then prevailing view that the shark pallium was entirely olfactory (Herrick, 1948; Johnston, 1911). It was proposed that rather than non-olfactory modalities invading the pallia in amniotes as previously thought, multimodal neurons in "simpler" vertebrates evolved into unimodal and more specialized forms in "advanced" vertebrates - the parcellation theory (Ebbesson, 1980a; Herrick, 1948). They showed the presence of a "neocortical equivalent" region in the shark pallium, which received non-olfactory fibres from the thalamus which were substantiated by physiological and behavioural results (Cohen et al., 1973; Graeber et al., 1973). With regard to cell types and cytoarchitecture of the pallium, much less is known; the telencephalon in sharks consists of a mass of neurons and the pallial regions do not appear to show lamination (Smeets, 1983). However, it is worthy to note that in large brained and well-developed sharks as well as rays (Schluessel, 2015; Yopak et al., 2010), there is evidence of complex behaviour and cognitive abilities. The information available in elasmobranchs as a whole is limited and more so, have not been investigated with more modern anatomical, physiological and transcriptomic approaches. Given the phylogenetic importance of elasmobranchs in that their common ancestor could reveal features from which all gnathastomes (jawed vertebrates) emerged, there is a clear need for further study.

7.1.4 Amphibians

Amphibians, also anamniotes, do not appear to have a laminated cortex and essentially have a periventricular neuronal layer and a superficial layer rich with fibres, and the neurons do not seem to migrate and remain largely around the ventricles (ependyma, (Dicke and Roth, 2007; Herrick, 1948; Northcutt and Kicliter, 1980). These neurons around the ventricle usually have pear shaped somata with several (3-4) primary dendrites extending to the superficial layer

(Dicke and Roth, 2007). Several pallial areas with intrinsic and extrinsic connectivity have been identified including a proposed dorsal pallium or neocortical homologue as well as a rostral cortex which is an area proposed as the equivalent of the mammalian frontal cortex based on the anatomical location. The neurons in the dorsal and medial pallium tend to be more spiny than the neurons in other pallial regions (Roth et al., 2007). In terms of connectivity, the medial and ventral pallium are known to project to the hypothalamus, preoptic area and ventral thalamus. Surprisingly, the dorsal pallium does not appear to have extratelencephalic projections (Roth et al., 2007). In terms of input, the dorsal thalamus and the ementia thalami are known to project to the pallial areas (Karamian et al., 1966; Laberge and Roth, 2007). At least two divisions in the dorsal pallium have been proposed with the dorsal subdivision thought to receive visual and somatosensory inputs and the ventral part receiving olfactory input (Laberge and Roth, 2007; Vesselkin et al., 1971). The medial pallium is known to receive thalamic input (Northcutt and Ronan, 1992; Veselkin and Ermakova, 1978) and seems to show multimodal responses including visual, somatosensory and auditory responses (Karamian et al., 1966; Mudry and Capranica, 1980; Northcutt and Ronan, 1992). Yet again, several scattered reports exist on the amphibian pallia and more studies with modern techniques are clearly required.

7.2 PALLIA IN AMNIOTES

7.2.1 Non-avian reptiles

7.2.1.1 Lateral cortex (Olfactory cortex)

The lateral cortex in reptiles is the largest olfactory cortical region receiving input from the mitral cells in the olfactory bulb through the lateral olfactory tract (LOT), along with the smaller olfactory tubercle and the diagonal band nuclei (Reiner and Karten, 1985; Ulinski, 1990). There is relatively more information about the reptilian olfactory cortex in snakes than in other reptiles. Like the mammalian piriform cortex, the lateral cortex of reptiles is three-layered. In snakes, the outermost layer consists of stellate cells, which have dendrites confined to layer 1. The lateral cortex has two divisions - rostral and caudal. Layer 2 of the rostral lateral cortex consists of distinctive “double-pyramidal” cells (two apical dendrites) called bowl cells (Ulinski and Rainey, 1980). These cells have axons descending into layer 3, which continue as efferent projections. In the caudal lateral cortex, the principal cells are more pyramidal-like with the cell body in layer 2 or 3 and an apical dendrite traversing to layer 1.

Clearly, similarities in connectivity of the lateral cortex and those of the mammalian piriform cortex can be observed (detailed under mammalian cortices). Lateral cortical projections targeting the contralateral lateral cortex have been shown in lizards (Martinez-Garcia et al., 1986), which cross to the contralateral side through the habenular commissure. These projections would be akin to corticocortical callosal projections of neocortex (Kim et al., 2015). Additionally, monosynaptic olfactory bulb inputs have been shown to terminate in the

contralateral lateral cortex in turtles, also crossing in the habenular commissure. However, this is in contrast to the mammalian olfactory bulb projections, which target only ipsilateral olfactory cortices (Orrego, 1961; Ulinski, 1990). Lastly, in the turtle, there are relay nuclei, which receive input from the olfactory bulb and project to the lateral cortex – the diagonal band nuclei and the nucleus of the LOT (Martinez-Garcia et al., 1986). In mammals, the anterior olfactory nucleus serves as a relay from the olfactory bulb to the piriform cortex (McGinley and Westbrook, 2011).

7.2.1.2 Dorsal cortex (Neocortical homologue)

In the hunt for finding the evolutionary precursor for the mammalian neocortex, studies in the amniote turtle cortices have been of importance. The cortex in non-avian reptiles can be divided into medial, dorsal and lateral cortices. The dorsal cortex is considered to be the homologue structure of neocortex based on its anatomical location, between the olfactory cortex and hippocampus in reptiles (Connors and Kriegstein, 1986; Laurent et al., 2016b; Smith et al., 1980). This three-layered cortex is located above the nuclear DVR (dorsal ventricular ridge, (Ulinski, 1983). The intrinsic lamination of the turtle dorsal cortex is, however, different from the mammalian six-layered neocortex in that there are three distinct layers (Connors and Kriegstein, 1986; Laurent et al., 2016b). The DVR is a nuclear pallial structure whose homology is still debated and unclear (Briscoe et al., 2018; Briscoe and Ragsdale, 2019). Most investigations of the dorsal cortex have been performed in the turtle, whose dorsal cortex is said to correspond to the visual cortex of mammals, although the possibility that it can process other sensory modalities as well is acknowledged (Connors and Kriegstein, 1986; Fournier et al., 2018; Hemberger et al., 2016; Laurent et al., 2016a; Naumann et al., 2015). There are also similarities in the afferent projections of the dorsal cortex with those of the neocortex in terms of thalamic inputs and modulatory inputs, including adrenergic and cholinergic inputs (Desan, 1981; Hall et al., 1977; Hall and Ebner, 1970; Smith et al., 1980). Both neocortex and the dorsal cortex have extrinsic projections, which target the brainstem and thalamus although the extrinsic projections of the DVR are less well known (Hall et al., 1977; Ulinski, 1986). It is noteworthy, however, that corticotectal (corticocollicular in mammals) projections have so far not been shown in turtle dorsal cortex but have been demonstrated in lizards (Elprana et al., 1980). However, in a more recent study identified based on select marker expression, IT type neurons in the alligator dorsal cortex and DVR as well as in the avian Wulst and DVR (Briscoe et al., 2018).

There are two main classes of neurons in the turtle dorsal cortex; pyramidal cells that form the main output of the cortex, and stellate interneurons (Northcutt, 1970). The pyramidal cells have long, spine-bearing apical dendrites that ascend to the molecular layer (outermost layer devoid of neurons), and basal dendrites extending into a subcellular zone (Smith et al., 1980). The pyramidal cells are concentrated in layer 2 and are immunopositive for glutamate (Connors and Kriegstein, 1986). However, unlike neocortical pyramidal neurons, most turtle “pyramidal

neurons" are characterised by multiple apical dendrites. The basic electrophysiological properties of turtle dorsal cortical pyramidal cells, however, resemble their neocortical pyramidal counterparts (Connors and Kriegstein, 1986; Hemberger et al., 2019; Shepherd, 2011). Interestingly, burst firing in pyramidal neurons has not been observed in turtle dorsal cortex, which is a classical feature of neocortical layer 5 neurons and hippocampal neurons (Kandel and Spencer, 1961; Larkum et al., 2008; Masukawa et al., 1982; McCormick et al., 1985). However, the turtle hippocampal cortex has burst firing neurons (Shen and Kriegstein, 1986). The stellate cells of their dorsal cortex are aspiny GABAergic cells present in the molecular or subcellular layers and are considered the equivalent to the GABAergic neocortical aspiny stellate cells (Blanton et al., 1987; Connors and Kriegstein, 1986). The turtle dorsal cortex also has substance P-expressing neurons in layers 1 and 2 (Brauth et al., 1983). A small number of neurons have been found that are immunopositive to somatostatin as well (Bear and Ebner, 1983).

In the turtle dorsal cortex, layer 4 - and 5 - like cell types were identified based on select molecular marker expression (Dugas-Ford et al., 2012). They were found to form distinct but partially overlapping "fields", which are present in the middle excitatory cell layer (Dugas-Ford et al., 2012). However, in a more recent high throughput study of marker expression in the turtle dorsal cortex showed an overlap of neocortical layer 4 and layer 5 markers in a single cell subpopulation. There was no evidence for a "one-to-one" homology of dorsal cortical and neocortical cell types (Tosches et al., 2018). However, there was clear homologies between the interneurons in that the main "classes" of GABAergic interneuron subpopulations like somatostatin, parvalbumin and VIP expressing GABAergic interneurons were homologous to the neocortical interneuron subpopulations (Tosches and Laurent, 2019; Tosches et al., 2018).

There has also been analysis of the thalamic input, which is the main sensory input coming into the turtle visual cortex. The thalamic afferent input comes in through the molecular layer, unlike that in the mammalian neocortex, but similar to the piriform/hippocampal cortices. The pyramidal cells in the turtle dorsal cortex were found to be excited by thalamic input and inhibited by GABAergic feedforward, as well as feedback inhibition (Connors and Kriegstein, 1986; Hall et al., 1977; Shepherd, 2011; Smith et al., 1980). The axons of these pyramidal cells exit the turtle visual cortex to traverse via subcellular areas (layer 3), similar to the neocortical layer 5 output neurons. Basic circuit modules of the turtle dorsal cortex have been proposed similar to that of the neocortex. These modules have been proposed as the evolutionary precursor of the canonical microcircuits of the neocortex (Shepherd and Rowe, 2017).

Sensory input relayed via the thalamus to pallial structures have been known for a long time in non-avian and avian reptiles, but more so in avians. In turtle, the visual input from thalamus is relayed via the lateral geniculate nucleus (LGN). It has been shown that the retina projects bilaterally and topographically to the LGN (Bass and Northcutt, 1981a, b; Hall and Ebner, 1970). However, the turtle dorsal cortex does not receive a topographic input from the LGN

(Mulligan and Ulinski, 1990; Ulinski, 1990). The visual relay from the thalamus targets the dorsal cortex and a more recent study showed that this was not organized retinotopically (Fournier et al., 2018). Other than the retinal relay via the lateral geniculate, trans-tectal visual input reaches a distinct part of the DVR (Benowitz and Karten, 1976; Hall et al., 1977; Karten et al., 1973; Pritz, 1975). Somatosensory input also targets a discreet part of the rostral dorsal cortex (Korzeniewska and Güntürkün, 1990; Reiner, 1993; Wild, 1997; Wild, 2015), however, the organisation of the somatosensory and motor areas in the dorsal cortex is not clear (Laurent et al., 2016a). There is a need for more detailed studies on sensory organisation in the pallia of non-avian reptiles, not only in terms of more detailed anatomy and physiology but also sampling in different reptilian species.

7.2.2 Avians

Research on the avian pallia has been instrumental in shaping ideas on cortical evolution. As mentioned before, early work from Karten provided a circuit and cell type framework for cortical evolution (Karten, 1969). This proposal of input, output and associational cell type and circuit perspective has found some recent molecular evidence in terms of specific marker expression (Dugas-Ford and Ragsdale, 2015; Dugas-Ford et al., 2012). It is interesting to note that birds have large brains in relation to their body size with number of neurons in the telecephalon matching and with densities equivalent to that of primates (Olkowicz et al., 2016; Sol et al., 2008). Furthermore, the cognitive abilities of birds match primates, with tool-using and problem-solving capability and even extending to recognition of the self and communication (Balakhonov and Rose, 2017; Emery and Clayton, 2004; Massen et al., 2014; Raby et al., 2007; Smirnova et al., 2015). The avian telencephalon does not have any laminated structure like the neocortex and thus represents the embodiment of intelligence without a neocortex (Güntürkün, 2005; Maler, 2018). Only the Wulst displays a degree of lamination (Watanabe et al., 1983). The bird pallium has clusters of cells or nuclei which are divided into two main areas – the DVR and the Wulst, with the DVR further divided into the mesopallium, nidopallium and arcopallium (Jarvis, 2009; Watanabe et al., 1983; Yamashita and Nomura, 2017). The leading hypothesis for homology between the avian pallia and the neocortex in is that layer specific neurons of the neocortex are organized into nuclei in birds (Briscoe et al., 2018; Briscoe and Ragsdale, 2018a; Dugas-Ford and Ragsdale, 2015; Dugas-Ford et al., 2012). The mesopallium and nidopallium have been shown to have neurons with mammalian IT-like marker expression with the speculation that the nidopallium could have neocortical layer 4 like cells given that at least the sensory areas of the nidopallium express layer 4 markers (Briscoe and Ragsdale, 2018a, 2019). The arcopallium is also known to send projections to the thalamus, brainstem and rostral spinal cord (Dugas-Ford and Ragsdale, 2015; Karten et al., 1973; Zeier and Karten, 1971). Similarly, brainstem projecting neurons in the Wulst have been proposed with specific marker expression as neocortical layer 5 cells. Some of these cells in the Wulst are known to send projections to the rostral spinal cord in some species (Reiner and Karten, 1983; Wild and Williams, 2000). Thus, neocortical layer 4 equivalent input cells, associational

intratelencephalic cells and output layer 5-like cells have been described as nuclei in the avian pallium (Briscoe et al., 2018; Dugas-Ford et al., 2012).

Other than cell types and connectivity, another strong line of argument for homology in avians has been on distinct sensory channels targeting pallial structures. Different sensory modalities are known to target both the Wulst and the DVR in birds relayed via nuclei in the thalamus. The visual input consists of both major pathways known in mammals – the retinal input relayed via the lateral geniculate to the anterior Wulst and the trans-tectal visual input is relayed to the DVR via the nucleus rotundus of the thalamus (Hunt and Kunzle, 1976; Karten and Hodos, 1970; Karten et al., 1973). Furthermore, there seems to be some degree of retinotopy in the visual Wulst of the Zebra Finch (Keary et al., 2010; Michael et al., 2015). Other than the visual Wulst, there is also a discrete representation of somatosensory information in the posterior Wulst, which is relayed via the somatosensory classical pathway as seen in mammals with the information from the dorsal root ganglion relayed to a dorsal column nucleus which is then relayed to the dorsal intermediate ventral anterior nucleus of the thalamus which projects to the posterior Wulst (Wild, 1997; Wild, 2015; Wild and Williams, 2000). In addition, auditory input is also relayed via the thalamus to the DVR (Wang et al., 2010). Distinct sensory channels thus seen in avians with similarities to the mammalian thalamocortical sensory pathways have contributed to proposals of homologies of the mammalian neocortex with the avian Wulst and DVR (Briscoe and Ragsdale, 2018a; Dugas-Ford and Ragsdale, 2015).

7.2.3 Mammalian cortex

7.2.3.1 The piriform cortex

Of the three types of the cerebral cortex, the phylogenetically older cortex is the olfactory cortex, also called the *paleocortex* (Haberly, 1998). The three-layered olfactory cortices have been considered as relatively simpler model systems to analyse sensory processing than the more elaborate six-layered neocortex (Shepherd, 2011). This notion has found support in a number of studies, which have shown similarities in the organisation of their local microcircuits and inherent connectivity.

The entire piriform cortex is three-layered. The outermost layer or layer 1 is called the *plexiform layer* that mostly contains incoming afferent fibres, but also dendrites and a small number of neurons. Notable neurons here are the large horizontal GABAergic cells unique to layer 1 (Suzuki and Bekkers, 2010; Young and Sun, 2009). Layer 1 is divided into two parts, layers 1a and 1b. The superficial layer 1a receives monosynaptic input from the olfactory bulb, through the LOT. The deeper layer 1b receives associative fibres from piriform and other olfactory cortices (Haberly, 1998; Price, 1973). Layer 2 consists of two divisions with the outer part called layer 2a, consisting of excitatory semilunar cells which lack basal dendrites, while the underlying layer 2b consists of superficial pyramidal cells (Haberly and Price, 1978). GABAergic neurons in this layer are small multipolar and bitufted cells. Layer 3 consists of

the deep pyramidal cells and the large multipolar cells, which are excitatory, and the sparsely spiny GABAergic large multipolar cells (Haberly, 1998; Haberly and Price, 1978). The deep pyramidal cells along with the superficial pyramidal cells are the principal neurons, which have morphologies similar to pyramidal neurons of neocortex. They are characterised by a spiny primary apical dendrite, which travels to the plexiform layer. These neurons also have spiny basal dendrites. The other interesting excitatory cell group, the semilunar cells present in layer 2, generally has two spiny apical dendrites and no basal dendrites (Haberly, 1998). The morphology of semilunar cells is comparable to the reptilian olfactory cortical bowl cells. Piriform layer 3 contains the principal excitatory cells, unlike layer 3 of the turtle dorsal cortex.

In terms of GABAergic neurons, large multipolar cells are a major inhibitory cell group many of which are termed basket cells due to the 'basket'-like terminations of their axons onto pyramidal cells, along with multipolar neurons and bitufted cells. These cells mediate feedforward and feedback inhibition as well as lateral inhibition (Haberly, 1998; Tseng and Haberly, 1989a, b).

The major source of input to the piriform cortex is the LOT, which consists of the axons of the mitral and tufted cells (Haberly and Price, 1977). These myelinated axons spread all over the piriform cortex (Haberly, 1998). The piriform cortex also receives neuromodulatory input from the basal forebrain, hypothalamus and brainstem (Haberly and Price, 1978). In terms of efferents, the piriform cortex projects (direct projections of pyramidal cells) to the entorhinal and perirhinal cortices, prefrontal cortices and amygdala (Carmichael et al., 1994; Price et al., 1991). The piriform cortex in addition projects unidirectionally to the ventral striatum and the mediodorsal thalamus (Heimer et al., 1982; Kuroda et al., 1992; Shipley and Ennis, 1996).

7.2.3.2 Neocortex

The neocortex is the most recent evolutionary version of cortex, with a six-layered microcircuit architecture. While the neocortex in most mammals is six-layered, it is noteworthy that the interspecies variability in cortical lamination also amongst mammals is substantial, and some species like dogs have fewer layers in the frontal lobe (Defelipe, 2011). The neocortex can be divided into three major divisions. The granular cortex, which comprises the sensory cortices, consists of small neurons (granule cells) packed in the intermediate layers (layer 4). Premotor and motor cortices lack a clear layer 4 and form the other major subdivision called the agranular cortex. The third major division consists of regions with varying amounts of granule cells, the association cortici (Douglas and Martin, 2004). The principal excitatory cells in the neocortex are the pyramidal cells, which along with spiny stellate cells constitute about 70 – 80% of all cells, while the rest 20 – 30% of the cells are inhibitory (Markram et al., 2004). There are a vast variety of GABAergic subtypes in the neocortex in terms of morphology, expression of neuropeptides and calcium-binding proteins, axonal targets, and firing properties (Huang and Paul, 2019; Markram et al., 2004).

The layer 5 pyramidal cells are the output cells of the neocortex. The subcortically projecting pyramidal cells are located in layer 5B and are generally large cells like for instance the Betz cells of the motor cortex, which project to the spinal cord or like the Meynert cells of the visual cortex that project to the superior colliculus (Douglas and Martin, 2004). The other subgroup is the layer 5A pyramidal neurons, which project to the contralateral cortex and striatum, and are intratelencephalic cells (Clare et al., 2018; Kim et al., 2015; Reiner et al., 2010). These cells have a subpopulation that does not target the contralateral striatum but terminate exclusively in the contralateral cortex (Kim et al., 2015). The IT- and PT-type cells are layered as 5A/5B in rodents (Reiner et al., 2003). The rodent neocortical layer 6 also gives rise to extrinsic projections - particularly the efferent projection to thalamus, the function of which remains enigmatic (Molnar, 2019; Rouiller and Welker, 2000). The IT cells are however found in all layers of the neocortex and the layer 4 neurons are a major subclass of IT neurons which are the primary recipients of sensory thalamic input. The classical connectivity from the layer 4 cells to the upper layer 2-3 cells and the projections of the layer 2-3 cells to layer 5 cells is a classical connectomic feature of the neocortical microcircuit also called the "canonical" microcircuit (Douglas and Martin, 2004; Harris and Shepherd, 2015).

The pyramidal cells generally have a typical morphology and can be distinguished by these prominent features: a pyramid-shaped soma, an apical dendrite that traverses to the molecular layer whilst branching and several basal dendrites directed laterally or downward from the soma (Douglas and Martin, 2004). The apical dendrite terminates in layer 1 with a tuft of branches. All dendrites are covered with spines except for the most proximal segments arising from the soma. The axon originates from the base of the cell and travels downward giving off local collaterals and traverses to terminate in other cortical or subcortical structures. However, a variety of differences in morphology can be observed amongst pyramidal cells in different layers (DeFelipe and Farinas, 1992).

Layer 4 of sensory cortices receives the densest thalamic input, although thalamic input targets all layers. The spiny stellate cells, which are confined exclusively to layer 4 of sensory cortices, are the major receivers of thalamic input (Douglas and Martin, 2007; LeVay and Gilbert, 1976; White, 1989). These cells have a fundamentally different morphology compared to the pyramidal neurons in that they do not possess an apical dendrite but rather have multiple dendrites of similar length emanating from the soma, giving them a 'star'-like appearance. Pyramidal and GABAergic neurons are the other recipients of thalamic input although to a lesser degree than the stellate cells (Douglas and Martin, 2004; Douglas and Martin, 2007; Hersch and White, 1981).

The thalamic projections are order-preserving projections in that the topography of sensory input to thalamus is maintained in cortex. Sensory representation is an innate feature of sensory and motor cortices, which preserve topography, although the representation degenerates in "higher"-order association cortices. One of the features of the thalamic relay is that the extent

of representation in a sensory cortex is maintained, as determined by the receptor density at the sense organ (Douglas and Martin, 2004). For instance, in the visual cortex of primates, the receptor density is highest in the fovea of the retina, which has the largest representation in the primary visual cortex (Kaas and Collins, 2001; Kaas and Lyon, 2001). In the cat visual cortex, the first order neurons which receive monosynaptic thalamic input, i.e the layer 4 cells, receive an input maintaining visuotopic mapping from the LGN (Ferster et al., 1996). There is also a partial segregation of geniculate afferents driven by the left and the right eye. Similar mapping can be observed in the somatosensory cortex wherein the hand and face receive the highest representations having the highest receptor densities. The barrel cortex in rodents has an individual representation for each whisker, with thalamic clusters relaying input from each whisker (Petersen, 2007). Distinct sensory and motor areas along with sensory specificity have been found in the neocortex of all mammals examined and is acknowledged to be a conserved ancestral feature which was present in the last common ancestor of all extant mammals (Kaas, 2013; Raghanti, 2017).

7.2.4 Human Neocortex

The evolutionarily most recent version of the neocortex is the human neocortex. While viewing an intact human brain, the overwhelming impression is from the large folded neocortex. A more detailed review of the human neocortex is beyond the scope of this thesis and I only touch very briefly on some evolutionary implications. The human neocortex is virtually identical in terms of primary sensory areas and their relative configuration and lamination with that of all other mammals. The human neocortex is much larger due to generation of a greater number of neurons which are caused by human -specific gene duplications during development (Fiddes et al., 2018; Suzuki et al., 2018). There is also consequently, an increase of upper layer 2-3 neurons which is also seen across mammals with increase in the neocortical surface area (Kalebic et al., 2018). Despite these differences, clear homologies in cytoarchitecture, cell types and sensorimotor organisation dominate neocortices across mammals unequivocally establishing a common ancestry of the human neocortex with the neocortices of other mammals (Kaas, 2013; Molnár and Pollen, 2014).

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